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An International Journal of Genetics

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1865

STUDIES ON *PERIPLANETA AMERICANA*
I. EXPERIMENTAL ANALYSIS OF MALE MEIOSIS

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1. INTRODUCTION

THE main cytological features of the american cockroach (*Periplaneta americana*) have been described by Morse (1909) and more particularly by Suomalainen (1946). The diploid number of the species is 33 ♂ 34 ♀ with an XO/XX type sex chromosome mechanism. The male meiotic sequence is of particular interest in relation to the problems of spindle development, bivalent association and chromosome movement. It is anomalous in the following respects :

(i) The development of a polarised pachytene.
(ii) The absence of visible chiasmata at all stages of meiosis.
(iii) The complete absence of diplotene-diakinesis.
(iv) The occurrence of a pre-metaphase stretch following spindle development at the end of pachytene.

A similar division sequence has been recorded for some mantids (White, 1941 ; Hughes-Schrader, 1943*a*, 1950), but all previous work has been concerned solely with the descriptive aspects of the sequence, no attempt having been made to analyse these anomalies experimentally. The present paper is concerned with such an analysis by the induction of *c*-meiosis. Additional information has also been obtained from the study of spontaneous diploid interchange heterozygotes.

2. MATERIAL AND TECHNIQUE

Material for the present study was obtained from wild populations established in the following localities.

(i) Deep Navigation Colliery, Treharris, Glamorgan (T).
(ii) Wyndham Colliery, Ogmore Vale, Glamorgan (W).

For cytological analysis the testes of male imagines were dissected under saline (Baker, 1950), fixed in 1 : 3 acetic-alcohol and permanent squash preparations made in either acetic-orcein or aceto-carmine. Certain of the individuals were pre-treated for varying time periods by injecting solutions of *c*-mitotic inductors into their hæmocœls between abdominal tergites four and five (table).

Each of the paired testes consists of numerous follicles which are small and globular. Each follicle is divided into a series of zones in which the sex cells are at distinct, though locally synchronised, stages of development. As there is only localised synchronisation of division, the treatment times outlined in the table limit only the maximum, and do not define the effective duration of treatment.

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3. OBSERVATIONS

(I) *Normal meiosis*

Previous workers have established the salient features of the meiotic sequence but their accounts require extending and correcting in certain respects. It is necessary, therefore, to summarise the course of male meiosis up until the organisation of the first metaphase plate :

(i) Leptotene and zygotene are normal and there is no indication that zygotene pairing is in any way localised. At early pachytene the paired chromosomes are distributed at random throughout the nucleus.

(ii) The polar organisers commence their activity during mid-pachytene and all the chromosome ends, though euchromatic, respond to this polarity resulting in a double boquet-chromosome arrangement (plate, fig. 1).

TABLE

Schedule of treatments

C-mitotic inductor	Saline solution form injected	Treatment time
Alpha-bromo-naphthalene	Saturated	2 hours
Coumarin	,,	2 hours
Para-dichlorobenzene	,,	4½ hours
Colchicine	0·05 per cent.	1 ; 2 ; 4½ ; 10½ hours 3 days

(iii) Paired chromosomes retain a close parallel association until the end of pachytene but contract appreciably (plate, fig. 10). At this stage separation of homologous chromosomes is localised at the centromere and the nuclear membrane is still intact.

(iv) On the dissolution of the nuclear membrane at the end of pachytene a spindle is organised in the nuclear area. The bivalents orientate on this spindle but do not congress to a metaphase plate. Instead there is a rapid and asynchronous polar movement of the centromeres with a consequent separation of homologous chromosomes (plate, fig. 2). This separation proceeds smoothly and progressively from the centromere but terminally the bivalents remain in association so that they become attenuated, often to extreme degrees, in a pre-metaphase stretch (plate, fig. 3).

(v) With the exception of the single X chromosome, which is positively heteropycnotic and behaves as a univalent, the chromosomes stain feebly during the initial stage of the pre-metaphase stretch presenting a diffuse or " lampbrush " appearance (plate, fig. 2). This condition is similar to that in other species of Orthoptera (*cf.* Hsu, 1948, and Srivastava, 1954), but has not been previously recorded for *P. americana*.

(vi) The pre-metaphase stretch is followed by an asynchronous re-approach of homologous centromeres, the congression of bivalents and the consequent organisation of a metaphase plate (plate, figs. 4 and 5). On recontraction the bivalents assume either a rod or ring type of configuration.

(ii) *C-meiosis*

The course of meiosis is considerably altered following the treatments summarised in the table. All the treatments produce the same general effects on the division sequence :

(i) Late pachytene bivalents are morphologically similar to those observed in untreated material and the homologues are closely associated except for localised centromeric repulsion. There is, however, no indication of polar activity.

(ii) On the dissolution of the nuclear membrane no spindle is organised, but the continued operation of repulsion in the centromeric loop results in the opening out of the bivalents until they remain associated terminally, rarely subterminally, either as rings or rods. This is accompanied by their progressive contraction (plate, figs. 6, 7 and 8).

(iii) In the absence of a spindle the bivalents remain unorientated and dispersed so that this stage resembles diplotene-diakinensis except for the absence of the nuclear membrane.

(iv) The highly condensed bivalents at " late diakinesis " have a pronounced tendency to clump, but even after three days' treatment with colchicine no indication of either restitution or pseudo-anaphase is observed, neither does any failure of pairing occur.

4. DISCUSSION

The problems of bivalent structure and chromosome movement implicit in the male meiosis of *P. americana*, apart from their own special evolutionary significance, are aspects of importance in relation to general chromosome mechanics. Although these have been discussed elsewhere (Cooper, 1949 ; Hughes-Schrader, 1950 ; Östergren, 1951 ; and Suomalainen, 1946), the present experiment makes a reconsideration of them desirable.

(i) *Bivalent structure*

Suomalainen (1946) argues that concealed chiasmata form the basis of the meiotic association in *P. americana*, supporting his argument by three principal observations :

(i) The occurrence of visible chiasmata in certain other species of roaches.

(ii) The occurrence of terminalisation resulting in the production of either ring or rod bivalents.

(iii) The regular formation of a ring multiple in an individual heterozygous for a single interchange.

Cooper (1949), however, maintains that these observations are all open to alternative interpretation. To the observations suggesting chiasma formation can be added :

(iv) The occasional observation of subterminal associations.

(v) The development of open cross bivalents (plate, fig. 6).

(vi) The persistence of terminal connections at the pre-metaphase stretch.

(vii) The regular formation of multiple associations in spontaneous interchange heterozygotes.

(viii) The relative constancy in the number of associated arms per nucleus at " induced diakinesis ". In interchange heterozygotes the proportion of ring to rod bivalents decreases with increasing multiple formation, the total number of associated arms remaining relatively constant.

The association frequency is thus similar to that which one might expect were the chromosomes held together by chiasmata.

Individually these conditions can occur in the absence of chiasmata, but collectively the indirect evidence favours chiasma formation at least on occasion. The rapidity of terminalisation, however, would indicate that where chiasma occur they are formed near the ends of the chromosomes. It seems best to conclude that while the indirect evidence suggests the occurrence of chiasmata in some bivalents, the absence of non-centromeric loops indicates that an additional mechanism has been evolved to supplement or replace the mechanical association afforded by chiasmata. Hughes-Schrader (1950) suggests that this mechanism consists of a prolongation of parallel pairing by lateral attraction. While lateral attraction may operate in *P. americana*, it is more probable that the association of homologues, initiated by specific zygotene pairing, is maintained by a general and non-specific stickiness of the matrix. Three lines of evidence support this suggestion:

(i) The centromeres are localised but, in addition to their association at the centromere, the pro-metaphase II chromatids are also held together at other regions (plate, fig. 9).

(ii) Occasional bridges are observed connecting non-homologous bivalents (plate, fig. 10).

(iii) In interchange heterozygotes, consequent upon failure of multiple formation, it follows that some of the " bivalents " must be incompletely homologous and therefore one would not expect a close parallel association of partner chromosomes along their entire length. Such a condition does, however, obtain and, since the interchanges generally involve long non-interstitial segments, torsion pairing can hardly account for such an association (plate, fig. 10).

These observations indicate the operation of a non-specific sticky

factor in maintaining chromosome association. During the stretch this matrical stickiness terminates: at maximum stretch and subsequent metaphase the meiotic association of bivalents is maintained solely by terminal affinity.

Summarising, it is suggested that, whilst chiasmata may be formed in *P. americana*, matrical stickiness and terminal affinity are the main factors which maintain the association of homologues.

(ii) *Chromosome movement*

Four independent forces have been suggested as contributing to the pre-metaphase stretch (Hughes-Schrader, 1943*a*, 1947):

(i) Centromeric repulsion.
(ii) Centrosome-centromere attraction.
(iii) Spindle elongation.
(iv) Half-spindle activity.

The first of these forces is operative during typical diplotene-diakinesis whereas the remainder are normally associated with the later meiotic stages. Obviously if the three latter forces could be suppressed experimentally then the pre-metaphase stretch would be largely inhibited and the continued action of repulsion in the centromeric loop might then be expected to result in the formation of diplotene-diakinesis type configurations. This expectation has been largely fulfilled. The main effect of *c*-inductors at meiosis in *P. americana* is comparable with that recorded by Levan in *Allium* (1939), namely an inactivation of the spindle apparatus. Spindle inhibition at *c*-meiosis has also been obtained in male *Chorthippus longicornis* (Coleman, 1947).

Östergren (1951), in discussing the subject of centromeric repulsion, is of the opinion that "there does not exist an action of this kind at any stage of mitosis and meiosis" and this despite evidence to the contrary (Hughes-Schrader, 1943*a*). There is no doubt from the present analysis that localised separation of homologues in the meiotic bivalent results from centromeric repulsion and is in no way connected with any centromere-centrosome attraction. Two observations support this contention:

(i) Separation of the centromeres takes place at a time when the nuclear membrane is still intact. At this stage while the telomeres respond to polar activity, the centromeres show no such response and the plane of separation of the bivalent bears no relation to the polarity of the nucleus (*cf.* Le Calvez, 1947).
(ii) Localised separation of the bivalents at the centromere takes place after *c*-induction treatment at a time when polar activity has been completely suppressed.

The movement of the centromeres during the pre-metaphase

stretch of *P. americana* is essentially similar to that which takes place at first anaphase. It differs from the latter, however, in two respects :

(i) It occurs immediately following pachytene.
(ii) It does not result in complete separation of the homologues.

From the mantid evidence, Hughes-Schrader (1950) has concluded that the absence of diplotene-diakinesis is in no way correlated with the pre-metaphase stretch. The present investigation shows that this is an over-generalisation.

Hughes-Schrader's own investigations support this contention. During the stretch phase in *Stagomantis carolina* (Virginia) the centromere is not the only locus of separation. Non-centromeric loops are also present which must result from generalised repulsion, or to use Hughes-Schrader's terminology, from a lapse of lateral attraction. This generalised repulsion is operative during typical diplotene-diakinesis and, in conjunction with centromeric repulsion, results in the opening out of the pachytene bivalents (Darlington, 1937). There is no reason to doubt that both these forces would continue to operate in the absence of a spindle as indeed they must do in most organisms, and that inhibition of the spindle in *Stagomantis* would result in the production of diplotene-diakinesis as it does in *P. americana*. Non-centromeric loops during the pre-metaphase stretch have also been described for *Tauromantis*, *Phyllovates* and *Stagomantis heterogamia* (Hughes-Schrader, 1950). It would appear, therefore, that all these mantids are comparable with *P. americana* : the absence of diplotene-diakinesis is due solely to the intervention of the pre-metaphase stretch.

Östergren (1951) has put forward two conditions on which the presence or the absence of the stretch depends :

(i) The maintenance of parallel pairing.
(ii) The degree of chromosome contraction prior to spindle formation.

The observations on *P. americana* support these suggestions. That there is a lapse of matrical stickiness is indicated by the fact that diplotene-diakinesis can be induced. Further the chromosomes, although contracted at late pachytene, become diffuse prior to their orientation. The pachytene stages of *Periplaneta* and *Callimantis* are comparable, but the difference in the subsequent behaviour of *Callimantis* can be attributed to the continued parallel pairing of the contracted chromosomes.

(iii) *Non-chiasmate meiosis*

It is now accepted that chiasmata are not an indispensable factor in maintaining the association of homologous chromosomes. This was first established by the classical work of Darlington (1934) on the autosomal bivalents of male *Drosophila pseudo-obscura* and has since

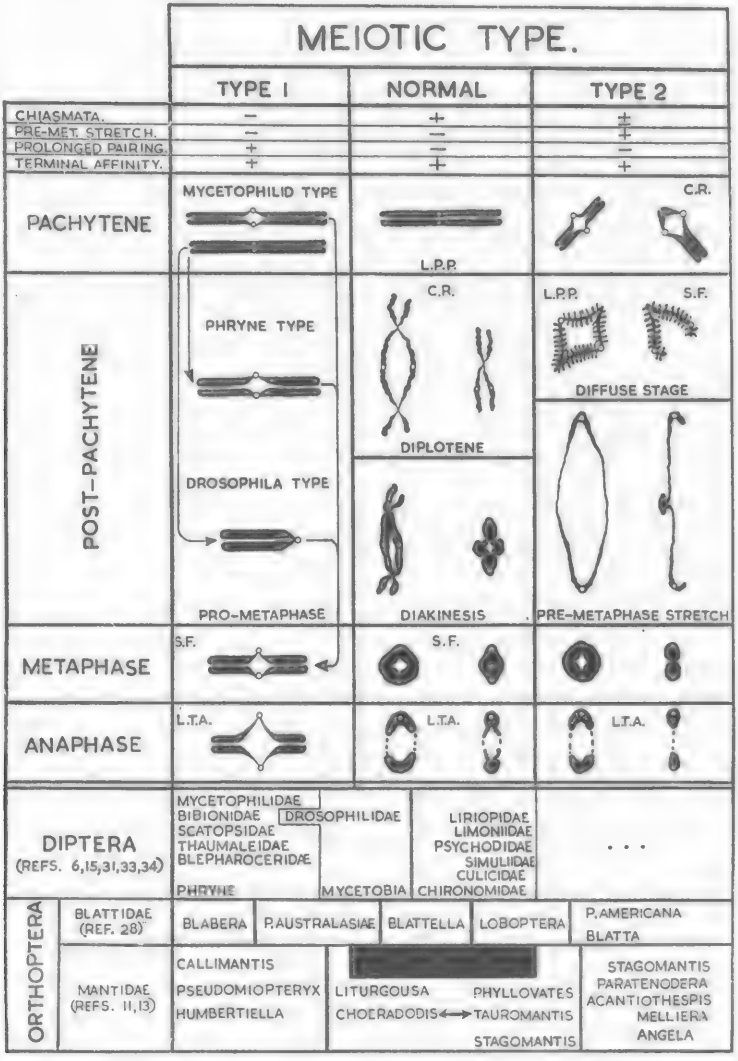

FIG. 1.—Schematic comparison of normal and non-chiasmate types of male meiosis.

C.R. = Centromeric repulsion : S.F. = Spindle formation.
L.P.P. = Lapse of parallel pairing : L.T.A. = Lapse of terminal attraction.

been confirmed by a number of workers on other species (Hughes-Schrader, 1943*b* ; Le Calvez, 1947 ; Piza, 1943 ; Wolf, 1941, 1950).

Meioses, where an alternative force either replaces or supplements chiasmata, are of two main types (fig. 1) ; types intermediate between normal meiosis and both of these variants are also known.

Type (1). In which parallel pairing is maintained until first anaphase. Here, homologues show no visible connection and chiasmata are clearly absent. The early repulsion of the centromeres may be suppressed as in *Drosophila*, *Blabera*, *Penthetria*, *Scatopse* and the mantids ; it may be evident during pachytene as in the mycetophilids, or during post-pachytene as in *Phryne* and *Thaumalea*. Finally, bivalents of the first and third categories may both occur in the same species as in *Periplaneta australasiae*. The observations on *Mycetobia* (Wolf, 1950), where bivalents with chiasmata occasionally show a persistence of parallel pairing, together with those on *Liturgousa* and *Choeradodis rhombicollis* (Hughes-Schrader, 1943*a*), where some bivalents are chiasmate whilst others are associated by lateral attraction, show stages intermediate between type (1) and the normal sequence.

Type (2). In which pachytene is followed by a pre-metaphase stretch which results in the opening out of the bivalents. Here there is no clear separation between homologous pairs except that localised at the centromere. The presence or absence of chiasmata in these cases cannot be established by observation. However, a number of mantid species (*Phyllovates impunctata*, *Tauromantis championi*, *Stagomantis heterogamia* and *Stagomantis carolina* (Virginia)), are intermediate between this type and normal meiosis : non-centromeric loops indicative of chiasma formation are apparent during the pre-metaphase stretch.

Normal meiosis has not been recorded in mantids, nor has the pre-metaphase stretch been found in dipterans. The mantids are, however, interesting in that they contain types intermediate between both extreme variants. The whole range of meiotic types from the normal to both extremes occurs only in the blattids. These considerations generally apply to only the autosomal bivalents.

The development of forces other than chiasmata capable of maintaining the meiotic association has permitted the independent evolution of a non-chiasmate meiotic sequence in several animal groups. Such a meiosis would tend to maintain gene sequences and would confer greater immediate fitness once favourable gene combinations had been built up. This may account for the evolution of this type of meiosis in outbreeding animals.

5. SUMMARY

1. In the normal meiotic sequence of male *P. americana* the spindle forms at the end of pachytene following polarisation of the nucleus.

2. Bivalent orientation is followed by a pre-metaphase stretch. Recontraction of the chromosomes with subsequent asynchronous congression leads to the formation of a metaphase plate.

Plate

Normal and *c*-meiosis in male *P. americana*. ×1800.

(Figs. 6, 7, 8 and 10 are taken from interchange heterozygotes which have the following chromosome formula—2.IV+12.II+X).

FIG. 1.—Mid pachytene, double polarisation.

FIG. 2.—Early pre-metaphase stretch, chromosomes diffuse, orientated but not congressed.

FIG. 3.—Pre-metaphase stretch, chromosomes highly attenuated.

FIG. 4.—Pro-metaphase, chromosomes in congression.

FIG. 5.—Metaphase, chromosomes condensed, orientated and congressed.

FIG. 6.—Early induced "Diakinesis", chromosomes diffuse and non-orientated (α-bromonaphthalene 2 hr.; individual T. 9).

FIG. 7.—Mid induced "diakinesis" chromosomes less diffuse (α-bromonaphthalene 2 hr., individual T. 1).

FIG. 8.—Late induced "diakinesis", chromosomes deeply stained and highly contracted (colchicine 1 hr., individual T. 23).

FIG. 9.—Pro-metaphase II, chromosomes with non-localised regions of association.

FIG. 10.—Late pachytene, with beginning of centromeric repulsion and non-homologous bridges present. Although this individual (T. 15) is heterozygous for two interchanges only one multiple has been formed in this nucleus. Two of the "bivalents" are therefore incompletely homologous yet all bivalents are closely associated along their entire length.

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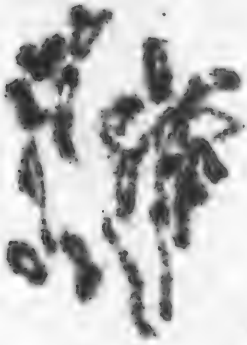

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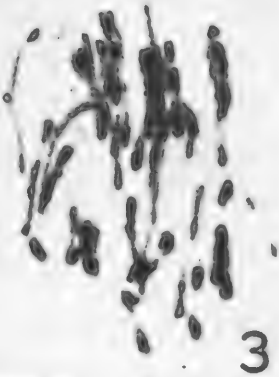

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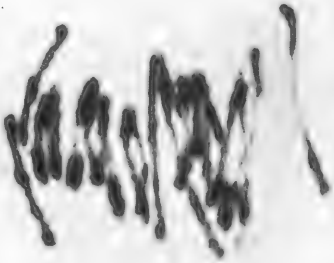

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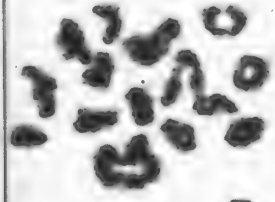

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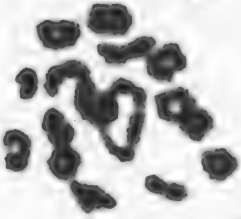

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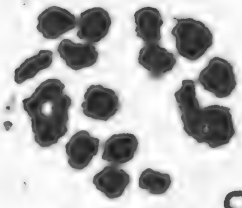

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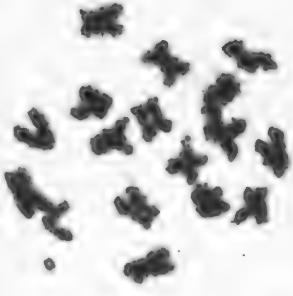

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3. The extreme pre-metaphase stretching of the bivalents is facilitated by a lapse of matrical stickiness and the diffuse nature of the chromosomes on their orientation.

4. Diplotene and diakinesis are suppressed by the pre-metaphase stretch. Injection of *c*-inductors results in the inhibition of the spindle and the absence of stretch. The continued operation of repulsion in the centromeric loop leads to the production of diplotene-diakinesis type nuclei.

5. The mechanical function normally performed by chiasmata is supplemented or replaced by matrical stickiness and terminal affinity. These forces are capable of maintaining the meiotic association in the absence of chiasmata.

6. The restriction of recombination, in the absence of chiasmata, may account for the establishment of non-chiasmate meiosis in outbreeding animals.

STUDIES ON *PERIPLANETA AMERICANA*
II. INTERCHANGE HETEROZYGOSITY IN ISOLATED POPULATIONS

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1. INTRODUCTION

INFORMATION concerning spontaneous structural changes in wild animal populations is extremely limited. This is particularly true of interchanges which appear to be rare in nature in the heterozygous condition. Of the recorded cases (*cf.* White, 1954) only Piza (1943 ; 1947*a*, *b* ; 1948*a*, *b*) and White (1940) have found established this type of structural hybridity.

Darlington and La Cour (1950) using interchanges as markers of ancestry have demonstrated the existence of a hybridity optimum and have also shown that a change from cross to self fertilisation favours the selective survival of ring forming progeny, that is of interchange heterozygotes. In the present account two isolated populations of the american cockroach (*P. americana*) are described in which imposed inbreeding has been accompanied by the establishment of interchange heterozygosity. *P. americana* has been introduced into this country by commercial traffic and populations of this organism have succeeded in establishing themselves only in a few restricted types of environment conditioned principally by temperature and humidity. The major habitat colonised by this species in South Wales has been the coal mine. These colliery populations all appear to have been established soon after the opening of the mines but within recent years the size of the populations has decreased.

2. MATERIAL AND TECHNIQUES

The investigation was made on male imagines obtained from three sources :—

(i) A wild isolated population established in The Deep Navigation Colliery, Treharris, Glamorgan. This mine is over 2000 feet deep and was sunk in 1873. Individuals from this source are pre-fixed T.

(ii) A mass laboratory culture set up and maintained from the wild Treharris population. This culture was started in 1946 by Mr G. T. Jefferson of the Zoology Department, Cardiff : since then additional specimens from the wild have been added to maintain its numbers. These specimens are pre-fixed NT or MT.

(iii) A wild isolated population established in The Wyndham Colliery, Ogmore Vale, Glamorgan. This mine, sunk in 1868, is approximately 1000 feet deep. The pre-fix W has been used for specimens from this locality.

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The induction of diplotene-diakinesis facilitated complete nuclear analyses which were otherwise difficult due to the pre-metaphase stretch and the compact metaphase plate. For cytological examination aceto-carmine or acetic-orcein squash preparations were made following prior fixation in 1 : 3 acetic-alcohol.

3. OBSERVATIONS

All the male imagines examined from the three sources gave typical chromosome counts at mitosis or meiosis II. The observation of multiple associations at meiosis I, however, showed that the populations were carrying a series of structural changes in the heterozygous condition (table 1).

TABLE 1

The incidence of interchange heterozygosity in seventeen specimens of P. americana

Population	Individual no.	No. of cells analysed	No. and type of multiple association
Treharris . .	T. 11	7	1 R.IV
	T. 16	15	
	T. 19	50	
	T. 9	15	1 R.IV–1 C.IV
	T. 23	50	
	T. 15	100	2 R.IV
	T. 2	13	2 R.IV+1 C.IV
	T. 1	100	3 R.IV
Laboratory . .	NT. 1	20	None
	NT. 2	40	
	MT. 18	100	1 R.VI+1 R.IV
	MT. 21	17	
	MT. 22	70	
Ogmore . .	W. 2	8	1 C.IV
	W. 3	25	
	W. 4	20	
	W. 1	11	2 R.IV+1 C.IV

(1) *Wild Treharris population*

Twenty specimens have been examined from this locality and all have been heterozygous for at least one interchange. Three independent interchanges is the maximum number observed in any one individual and no multiple association higher than four has been recorded.

(i) *Degree and type of multiple association.* The interchanged chromosomes are frequently associated in multiples (table 2). In T. 19, heterozygous for one interchange, a multiple was observed in 96 per cent. of the spermatocytes. Both the interchanged chromosomes for which T. 23 is heterozygous were associated in multiples in 72 per cent.

of the cells. T. 15, although of the same chromosome formula, is exceptional: the maximum of two multiples per cell was observed in only 4 per cent. of the cases. Maximum multiple association occurred in 58 per cent. of the spermatocytes in T. 1 heterozygous for three interchanges. Unpaired chromosomes and associations of three were not recorded in any individual.

Ring formation is frequent but closed associations are not invariably formed (table 1). Three interchanges, the maximum recorded for

TABLE 2

The percentage frequency of associations of four in six wild specimens of P. americana

No. and type of IV association	Frequency of types observed per cent.					
	W. 3	W. 4	T. 19	T. 15	T. 23	T. 1
None	24	15	4	17	8	...
1 C	76	85	2	...	8	1
1 R	...	...	94	79	12	3
1 R+1 C	...	...	...	1	72	17
2 R	...	...	...	3	...	20
1 R+2 C	...	...	...	...	...	1
2 R+1 C	...	...	...	...	...	54
3 R	...	...	...	...	...	4
Total cells	20	25	50	100	50	100
Chromosome formulae of individuals	1.IV			2.IV		3.IV

the population, were observed in closed associations in one individual (T. 1).

(ii) *Morphology of the multiples.* The three multiples observed in this population are of approximately equal size and similar morphology. In all cases:

(*a*) The pachytene crosses formed by associations of four chromosomes are roughly symmetrical (plate, fig. 1).

(*b*) The initial separation of homologous segments at late pachytene proceeds progressively from the centre of the cross: in bivalents it is initiated at the centromere.

All the interchanges are autosomal, the X chromosome of the male is invariably present as a univalent. Because of their similarity the multiples cannot be individually recognised with any certainty especially when they occur in different individuals.

(iii) *Orientation of the multiples.* Observations on the orientation of the multiples could be made only infrequently due to the closely packed metaphase plate. In the cells suitable for analysis both ring

TABLE 3

Percentage frequency of multiple associations in three laboratory bred specimens of P. americana

No. and type of multiple association	Frequency of types observed per cent.		
	MT. 18	MT. 21	MT. 22
None	...	5·9	...
1 R.IV	4	...	1·4
1 R.IV+1 C.IV	1	...	...
1 R.VI	43	17·6	5·7
1 R.VI+1 C.IV	30	70·6	...
1 R.VI+1 R.IV	22	5·9	92·9
Total cells	*100*	*17*	*70*
Chromosome formulæ of individuals	1.VI+1.IV+11.II+X		

TABLE 4

Frequency of disjunctional orientation in six wild specimens of P. americana

No. of associations of IV	Orientation				Observed frequency per specimen					
	Rings		Chains		W. 1	W. 2	W. 3	W. 4	T. 6	T. 11
	Dis.	N-D.	Dis.	N-D.						
0	...	...	...	...	...	...	6	3	...	2
1	1	...	...	...	5	...	...	...	2	3
	...	1	...	...	2	...	...	...	...	...
	...	...	1	...	...	8	17	17	2	2
	...	...	...	1	...	...	2	...	...	...
2	1	...	1	...	1	...	...	...	7	...
3	1	1	1	...	1	...	...	...	...	...
	2	...	1	...	2	...	...	...	...	...
No. cells analysed					*11*	*8*	*25*	*20*	*11*	*7*
Chromosome formulæ					3.IV	1.IV				
Proportion disjunctional chains					1·0	1·0	0·89	1·0	1·0	1·0
Proportion disjunctional rings					0·71	...	...	...	1·0	1·0

and chain multiples were invariably disjunctional (table 4). All the chromosomes at induced "diakinesis" and at metaphase lack interstitial associations. In bivalents homologues are associated terminally or rarely sub-terminally; in the multiples association is always terminal.

(2) *Laboratory population*

This population was started and has been maintained from the wild Treharris population. It is significant therefore that certain of the individuals from this culture are cytologically different from the wild specimens (table 1). Two individuals (NT.) were homozygous for all chromosomes (plate, fig. 3) while three (MT.) had a multiple association of six in addition to an association of four (plate, fig. 4).

(i) *Degree of multiple association.* The MT. individuals, although they are all of the same chromosome formula, vary appreciably in their degree of association (table 3). In all cases closed associations have been observed and in no instance have unpaired chromosomes or numerically uneven multiples been seen. In one individual (MT. 22) maximum multiple association was observed in 92·9 per cent. of the spermatocytes, the lowest recorded was 52 per cent. (MT. 18).

(ii) *Morphology of the association of six.* The pachytene configuration is star shaped and initial separation of homologous segments is located at the centre of the association (plate, figs. 5 and 6). Unfortunately, we do not yet have any information on the orientation of this multiple.

(3) *Wild Ogmore Vale population*

This population is considerably smaller than that at Treharris and only four individuals have been examined. All were structural heterozygotes (table 1). Three independent changes were observed in one individual (W. 1) and the highest multiple recorded was an association of four. As in the Treharris population only the autosomes are involved.

(i) *Degree and type of multiple association.* Multiples are formed less regularly than in the Treharris material. Nevertheless 76 and 85 per cent. multiple formation was recorded for W. 3 and W. 4 respectively, both of which are heterozygous for one translocation. In W. 1 only three of eleven cells analysed (*ca.* 27 per cent.) had maximum multiple formation. No ring formation was observed in the three individuals heterozygous for one translocation (W. 2, 3 and 4). In the fourth (W. 1) heterozygous for three changes, two of the multiples were observed in closed association.

(ii) *Morphology of the multiples.* Some of the multiples are morphologically similar to those described for Treharris (plate, figs. 7 and 8; text fig. 1*a*). One of the associations, however, is morphologically different. Here one of the chromosomes is much smaller than the others (plate, fig. 9; text fig. 1*b*).

(iii) *Orientation of the multiples.* The frequency of disjunctional

orientation is high but multiples do not invariably disjoin as they appear to do in the Treharris material. In W. 3 two of the nineteen chains of four were non-disjunctional and both of these were associated interstitially (plate, fig. 9 ; text fig. 1*b*). The three non-disjunctional ring multiples recorded for W. 1 were all observed at pre-metaphase and not after the organisation of a metaphase plate (plate, fig. 8 ; text fig. 1*a*).

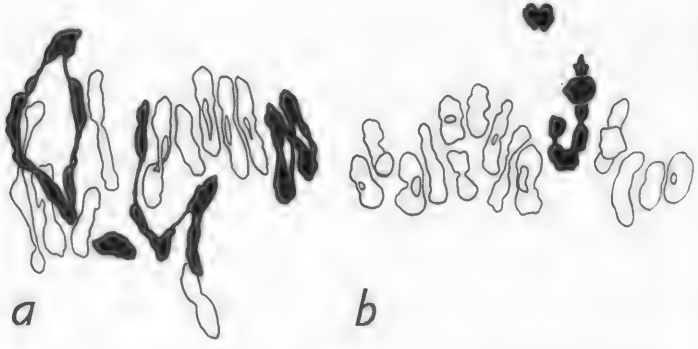


FIG. 1.

(*a*) Orientated pre-metaphase (2 R.IV+C.IV+10.II+X) ; one non-congressed discordant ring quadrivalent, one disjunctional chain quadrivalent and one disjunctional ring quadrivalent. Note asynchrony of congression (compare with plate, fig. 8).

(*b*) Metaphase I (C.IV+14.II+X) ; one non-disjunctional chain quadrivalent with an interstitial association. Note one short chromosome in chain (compare with plate, fig. 9).

In both figs. the quadrivalent associations and the single X chromosomes are shown solid. × 1800.

4. DISCUSSION

(1) *The nature of the structural changes*

Interchanges generally result in the production of closed multiple associations, there being few instances where they have occurred without subsequent ring formation (Burnham, 1932 ; Clarke and Anderson, 1935 and probably White, 1940). The cross-shaped pachytene configurations and the extensive formation of closed multiples in the Treharris material show that these structural hybrids are interchange heterozygotes. In the Ogmore material, however, three of the four individuals have only chain of four associations. The absence of rings cannot be due to any of the factors which would also limit the degree of multiple association because a high frequency was recorded in all three of these individuals. These chains of four include one very short chromosome. Therefore, while the loss of a fragment cannot be excluded, it seems more probable that this translocation is also reciprocal and that rings were not recorded because the interchange involved unequal segments.

(2) *The locus of breakage and reunion*

In all cases other than the asymmetrical chain of four, the basic chromosomes and their interchange derivatives are metacentric and approximately the same size. The pachytene crosses have approximately equal arms and separation of paired regions is initiated at the centre of the cross. It follows, therefore, that breakage and reunion have occurred near or at the centromere (*cf.* Morrison, 1953).

The slight inequality in arm length is more apparent in the pachytene association of six, three long arms alternating with three shorter ones. Since the chromosomes are approximately equal and metacentric the length of an interstitial segment is equal to only half the difference in length between adjacent arms and is therefore short. Further, the association of six is shaped like a star not a double obelisk; consequently the interchanges in the common chromosome (*i.e.* the one involved in two interchanges) must have occurred close together and the differential segments are also short.

(3) *Fertility*

The absence and limitations of interchange heterozygosity in animals can be explained by the fact that interchange hybrids may, as a result of non-disjunction, be subject to semi-sterility and therefore to negative selection (Wright, 1941). Segregation in an interchange heterozygote will depend upon:

(i) The degree of multiple association.
(ii) The orientation of the multiples.
(iii) The presence or absence of crossing over in the interstitial or differential segments.

In the interchanges under examination multiple associations are very frequently formed and no unpaired chromosomes or numerically uneven multiple associations have been found.

There is moreover a marked lack of randomness in the orientation of the multiples which is predominantly disjunctional. Disjunction in multiple associations depends essentially on the movement of adjacent centromeres to opposite poles. The probability of initial disjunction and its procession along a multiple is increased when the effective inter-centromeric distances are equal (Darlington, 1937; Östergren, 1951), a condition fulfilled when the chromosomes are equal-armed and associated only terminally (Darlington, 1937; Sax and Anderson, 1933). The present interchanged chromosomes tend to meet both these requirements; the rare non-disjunction at metaphase occurred only when the chromosomes were associated interstitially.

These two factors will facilitate disjunction but they are hardly adequate to account for the almost complete absence of non-disjunction. The operation of a third factor, the pre-metaphase stretch, is therefore suggested. The utility of the stretch phase in a functional diploid is

not clear but it is significant that although this phase is absent in certain mantids with an XO/XX sex chromosome mechanism, it is invariably present where multiple sex chromosomes of the X_1X_2Y type are involved (Hughes-Schrader, 1950). Again in the marsupial *Potorous tridactylys* a pre-metaphase stretch has been described analogous to that in the *Orthoptera* and here too there is a sex-chromosome association of three, the orientation of which is invariably disjunctional (Sharman and Barber, 1952).

That the stretch phase probably does influence co-orientation is indicated by the fact that in *P. americana* terminally associated multivalents have only been observed in non-disjunctional orientation at pre-metaphase, never at metaphase. This applies also to the sex trivalent in mantids studied by Hughes-Schrader (1943*a*, 1950), who suggests that the centromeres are capable of re-orientation after the stretch phase. This stage not only results in the terminalisation of association but also increases and tends to equate the inter-centromeric distances, all of which will increase the pliability of the configuration, a factor of prime importance in determining disjunction (Darlington, 1937). Further, the precocious organisation of the spindle at late pachytene increases the time available for centromeric interaction.

In animals, unbalance of the gametes does not result in hybrid incapacity in the same way as in plants since balanced zygotes can be produced by the fusion of complementary gametes (Darlington and Mather, 1949). Thus even cases of non-disjunction need not result in the production of non-functional gametes. This does not improve fertility, on the contrary it is disastrous because balanced eggs will fail to develop if fertilised by unbalanced sperm. This condition makes disjunction even more necessary in animals than in plants.

Finally, chiasmata when they are formed are almost certainly localised near the chromosome ends. The possibility of crossing over in the short interstitial and differential segments is thus remote.

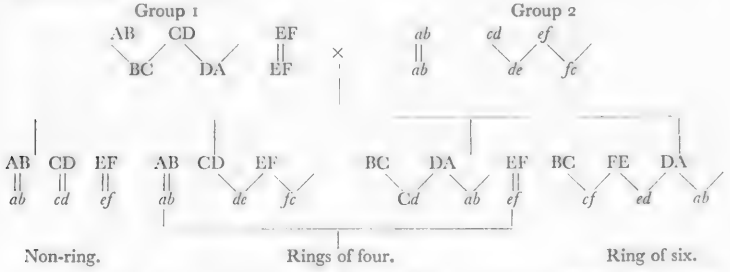
(4) *Selection of hybridity*

Ring and non-ring progeny from selfing a ring parent are sharply differentiated in genic as well as structural heterozygosity : a difference particularly pronounced with respect to the differential segments. Those from crossing with individuals with similar rings but dissimilar ancestry are not genically differentiated in anything like the same degree. In *Campanula*, artificially produced structural heterozygotes with ring formation show different degrees of true breeding in regard to ring formation according to the closeness of inbreeding. Structural homozygotes are less frequent in selfed progeny and more frequent in sister and non-sister progeny owing to the different degree of elimination of genic homozygotes (Darlington and La Cour, 1950). This principle of hybridity optimum, decisively demonstrated in *Campanula*, can account for the present position in *P. americana*.

The interstitial and differential segments of the present interchanges are very short. Genetically, however, a segment is also differential if it does not cross over and lies therefore between the centromere and the proximal chiasmata. Differential segments in this sense are a consequence of localised chiasmata and can occur in structural homozygotes. In *P. americana*, chiasmata, when they occur, are localised terminally and there are long proximal segments which do not cross over. Thus, although the interchanges have occurred near the centromere, the structural homozygotes and heterozygotes expected in the progeny of closely inbred interchange heterozygotes will be very different in their genic heterozygosity, those from cross breeding will not be.

The colliery populations must have arisen from small isolated units: they are therefore highly inbred. If there is an elimination of genic homozygotes, structural homozygotes will be few or absent from such populations. In fact they have not so far been found. The elimination of structural homozygotes from the progeny of wide crosses, however, is not expected. No controlled crosses have been made but the structure of the mass laboratory culture affords strong presumptive evidence that such structural homozygotes are not selected against.

The rings of six observed in the laboratory bred specimens have evidently been synthesised by the hybridisation of two interchange heterozygotes in which a common chromosome was affected: a synthesis similar to that described for *Pisum*, *Zea* and *Campanula* (Darlington, 1937). This method of origin can also account for the presence of structural homozygotes in the culture. The apparent absence of both these types in nature can then be explained on the assumption that the wild Treharris population consists of two spatially isolated mating groups: a suggestion supported by the distribution of roaches in the colliery. Representatives of both these mating groups have been brought together in culture and the cross may be represented as follows:



Thus the genic heterozygosity of the non-ring individuals need be little less than that of the ring progeny; hence their persistence in the laboratory population. That their non-observation in the natural

population is due to insufficient sampling or to the different pressure of selection in nature as opposed to the laboratory culture cannot, however, be excluded (*cf.* Darlington, 1939).

Suomalainen (1946) recorded one individual heterozygous for a single interchange in a sample of twenty individuals from a surface population in Berlin. It is possible, therefore, that the interchanges now fixed in the heterozygous condition were previously floating in the species and their occurrence is not due to the intra-specific hybridisation of geographically isolated races.

The extreme localisation, and in some bivalents the possible absence, of crossing over in the male *P. americana* will tend to maintain gene sequences. And interchanges by extending the linkage group will operate in the same direction. In this way the rival needs of fitness and flexibility can be reconciled in an outbreeding species. If, however, unnatural inbreeding is imposed upon such a concatenated system there will be a rapid increase in the frequency of homozygotes produced at fertilisation. Such genotypes will depart from the characteristic products of the outbreeding system and will not have been subjected to adjustment by selection. They will tend to be eliminated and thus a transition from outbreeding to inbreeding will favour the survival of structural heterozygotes.

5. SUMMARY

1. Two inbreeding populations of wild *P. americana* exhibit interchange heterozygosity producing rings and/or chains of four at meiosis.

2. All the male imagines examined are heterozygous for at least one interchange indicating that hybridity is near fixation level.

3. Generally the interchanges have resulted from breakage and reunion near the centromeres of autosomes.

4. Where individuals are carrying fewer interchanges than are known to exist in the population it has not been possible to determine whether they were homozygous for the basic or interchanged chromosomes.

5. The orientation of the multiples is predominantly disjunctional. It is suggested that the pre-metaphase stretch facilitates disjunction.

6. In a laboratory culture from one of the wild populations ring-of-six heterozygotes have been recorded. It seems probable that they have arisen by the mating of two heterozygotes in which a common chromosome was involved in different interchanges.

7. Complete structural homozygotes were also recorded in the laboratory culture. Their persistence is ascribed to their genic heterozygosity.

8. It is suggested that these karyotypes have not been found in nature because the population consists of two spatially isolated mating groups.

Plate

Interchange heterozygosity in male *P. americana*. ×2250.

FIG. 1.—Early pachytene, cross of four showing complete pairing of diffuse arms (T.15 colchicine 4½ hr.).

FIG. 2.—Induced "diakinesis" (2 R.IV+C.IV+10.II+X), X chromosome at 6 o'clock (T.1, α-bromonaphthalene 2 hr.).

FIG. 3.—Induced "diakinesis" (16.II+X), chromosomes diffuse (NT. 1, colchicine 2 hr.).

FIG. 4.—Induced "diakinesis" (R.VI+C.IV+11.II+X), bivalents showing strong contraction (MT.18, colchicine 4½ hr.).

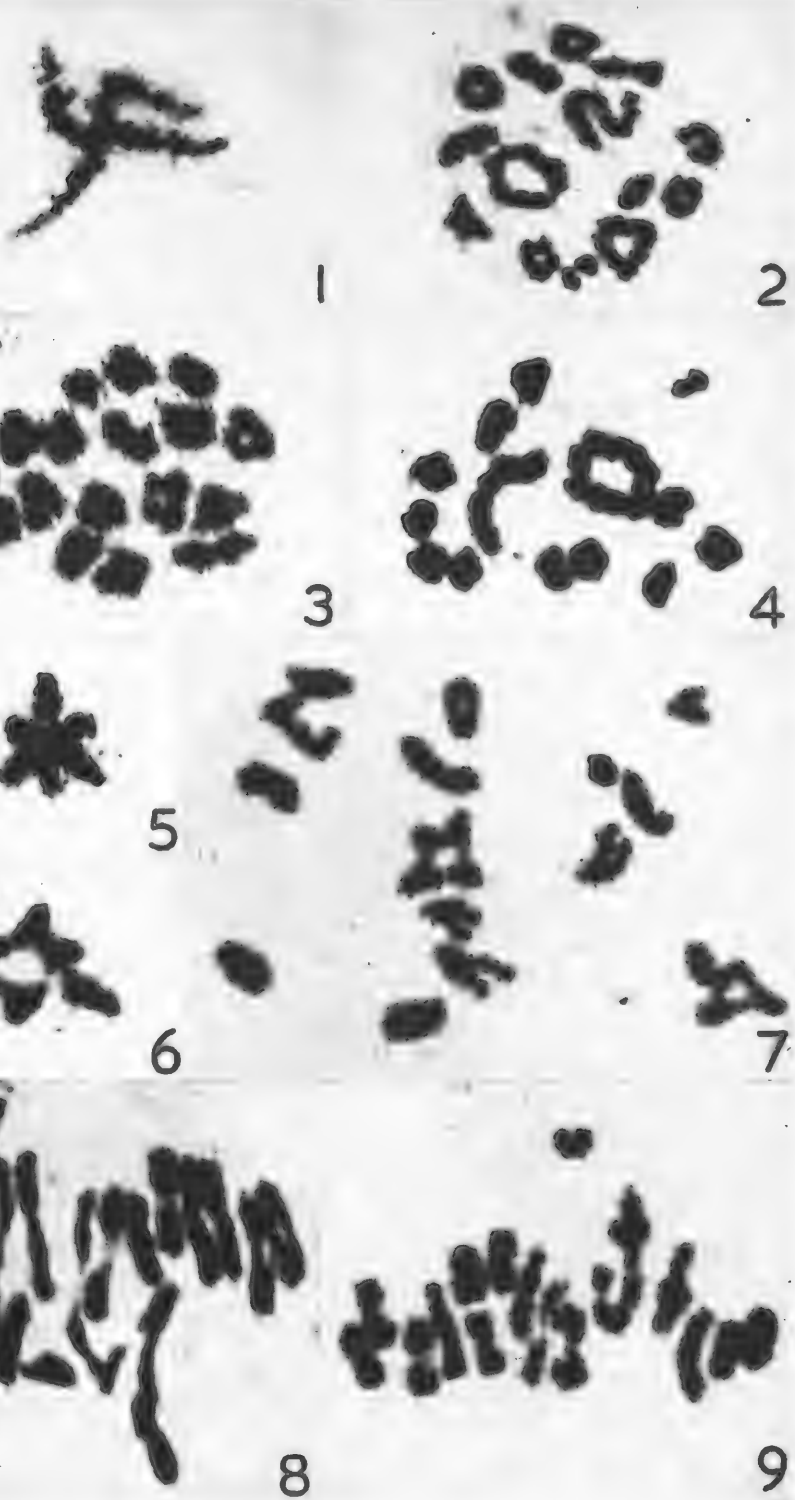
FIG. 5.—Mid pachytene, star of six chromosomes (MT.18, colchicine 4½ hr.).

FIG. 6.—Late pachytene, double cross of six chromosomes showing strong centromeric repulsion (MT. 18, colchicine 4½ hr.).

FIG. 7.—Late pachytene (2.IV+12.II+X), chromosomes showing centromeric repulsion (W. 1, untreated).

FIG. 8.—(2R.IV+1C.IV+10.II+X) Cell drawn in text fig. 1*a* (W. 1, untreated).

FIG. 9.—(1C.IV+14.II+X) Cell drawn in text fig. 1*b* (W. 3, α-bromonaphthalene 2 hr.).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9. The establishment of interchange heterozygosity is correlated with a change from outbreeding to inbreeding.

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EFFECT OF HIGH TEMPERATURE ON MEIOSIS IN *LOLIUM* : NUCLEOLAR INACTIVATION

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I. INTRODUCTION

Following their emergence from the resting stage, chromosomes begin to contract lengthwise and continue to do so throughout prophase, towards the end of which their short and cylindrical form is in marked contrast to the threadlike appearance earlier. The process of chromosome contraction, apart from its role in making them mechanically efficient, is considered important for the changes that it signifies in their chemical organisation, because of which the term " condensation " is generally used to describe it. Numerous studies of chromosome structure during various stages of the division cycle have helped to establish the fact that their linear contraction depends upon the assumption of a spiral form and that uncoiling at telophase restores the early prophase condition.

The degree of coiling of different chromosomes in the same complement is usually equal and constant, a fact which is responsible for their uniform thickness and constant lengths. Moreover the process of condensation is usually synchronised for all members of the complement so that at any particular stage in the nuclear cycle, they are found to be in similar states. Many exceptions, however, are known where particular chromosomes or parts of them do not keep step with the rest of the complement in their condensation cycle. These chromosomes or regions because of their differential behaviour were described as heterochromatic by Heitz (1931) who was the first to study them in great detail. The best known examples are the sex chromosomes in *Drosophila* and other insects, but autosomal regions in many organisms are found to be similarly affected (Darlington, 1937).

The investigations reported here concern experimentally induced differences in the degree of condensation of chromosomes which are members of the same complement and whose uniform and synchronised condensation is normally characteristic.

Experimentally induced changes of this nature bring to notice new characteristics of chromosomes, which may be of more than a limited interest. With the advances made in nuclear chemistry in recent years it has been possible to interpret purely morphological observations on chromosomes in terms of cytochemical changes. As well as contributing to a deeper understanding of chromosome

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behaviour, such correlated studies provide means of testing the chemical evidence which in the absence of absolutely reliable techniques is not always decisive. If this evidence is to be accepted, it should be possible to reconcile it with the observed behaviour of chromosomes. In a normal cell division, however, the co-ordinated changes in the nucleus obscure the individuality of chromosomes and their observed behaviour is not very revealing. Therefore, the need for inducing changes, which considerably increase the scope for the interpretation of cytochemical evidence.

2. MATERIAL AND METHOD

Population plants of perennial rye grass (*Lolium perenne* L.) which formed part of a collection for genetic studies provided the material. These have been clonally multiplied for a number of years and have given standardised controls. The treatment applied to induce the cytological changes, consisted of subjecting the potted plants to high temperatures before and during part of meiosis. The treatments were given in electrically heated and thermostatically controlled chambers.

Different temperature treatments have been tried, the successful ones being durations of 14, 38 and 58 hours at $35° \pm 2°$ C. followed by immediate fixation. Both fixation and storage were done in Carnoy to which a few drops of aqueous ferric chloride solution had been added. Staining was done mostly in acetocarmine, the other stain used being Feulgen which gave similar but not equally good results.

3. RESULTS

(i) *Mixed nuclei*

Meiosis in the perennial rye grass clones used is quite regular under normal conditions. All its 14 chromosomes, which is the diploid number, are found to be synchronised throughout the nuclear cycle, especially in respect of their prophase condensation which appears to be uniform for all members of the complement as also for all parts of individual chromosomes (plate I, fig. 1). Seven bivalents are regularly formed and these are co-ordinated in their congression and co-orientation on the metaphase plate. The nucleolus is equally regular in its formation. Study of the nucleus at earlier stages show that two nucleoli are organised in close association with two chromosomes and these fuse to form the single nucleolus found at later stages of prophase (plate I, fig. 2). It disappears shortly before the spindle is organised.

Following the three temperature treatments described earlier, the regular behaviour of chromosomes during meiotic prophase is visibly upset. The experimentally induced irregularities in the nucleus are as follows :

A very obvious change is manifested in the operation of a dual system in the prophase development of various members of the complement. The co-ordinated condensation of chromosomes in two different non-synchronised groups is particularly conspicuous, those in a normal group being far more contracted and deeply stained than the ones in the other retarded group. The differences, however,

are not always equally pronounced. In extreme cases chromosomes showing maximal condensation may be present along with others which have hardly undergone any spiralisation at all. In less extreme cases, diakinesis bivalents and those approaching diplotene may be found in the same nucleus (plate II, *cf.* figs. 1 and 6).

(ii) *Frequencies*

The abnormal behaviour in respect of condensation is evidently not characteristic of particular chromosomes in the complement since the number of affected chromosomes varies, as shown in table below, from cell to cell. Six fully condensed and one relatively uncondensed bivalent or two unspiralised chromosomes may be present in one pollen mother cell, while in another, the same number of chromosomes may be found in a reversed state of condensation (plate II, *cf.* figs. 1 and 9).

The following table compiled from observations on a random sample of 66 cells from two sister anthers shows the frequency of pollen mother cells with all their chromosomes normally condensed and others having varying numbers of condensed and relatively uncondensed chromosomes.

TABLE

Showing the variation in frequencies of PMC with retarded bivalents following treatment for 58 hours at 35°±2° C.

Chromosome complement . .	$2n = 14 = 7$ II						
Number of normal bivalents (with attached nucleoli)	1	2	3	4	5	6	7
Frequency of pollen mother cells (total 66)	*13*	*7*	*4*	*5*	*5*	*4*	*28*

N.B.—No normal cells with single normally organised nucleoli were found in this sample, although they occur perhaps in 1 per cent. of PMC.

The other temperature treatments give similar results with regard to the frequency of PMC's with varying numbers of condensed and uncondensed chromosomes.

Non-synchronised appearance of chromosomes such as described here may obviously result from two entirely different causes. Precocious development of some of the chromosomes or their failure to condense at the normal rate, would both produce the same phenotypic effect in the nucleus. The following observations clearly suggest failure of condensation and rule out the possibility of precocity in this particular instance.

(1) If the apparently retarded chromosomes were condensing at a normal rate, they should be able to reach their usual metaphase degree of condensation and catch up with the supposedly precocious

chromosomes towards the end of prophase. This they never do, thus indicating a failure on their part, which in some cases is very considerable, as already described.

(2) The fully condensed chromosomes are invariably present as bivalents and not as univalents. This would not be likely if the chromosomes concerned showed precocious condensation before pachytene, a fact which would be expected to interfere with their pairing and chiasma formation. Nor is there any evidence for a particularly rapid condensation of the chromosomes after pachytene ; the various stages leading to metaphase condition can often be seen.

The fact that the normally condensed chromosomes occur as bivalents and not as univalents is significant in more than one respect. It also shows that the homologous chromosomes respond to the temperature treatment in the same manner—both showing normal condensation or its failure.

It was pointed out earlier that the failure of condensation in the affected chromosomes may be complete or partial. The extent of this failure, however, varies.

In pollen mother cells in which a large number of chromosomes, as many as 5-6 bivalents, show normal condensation, the remaining few chromosomes appear almost completely unspiralised and hardly stain at all. It is usually obvious that the affected chromosomes have advanced little beyond their interphase condition. Occasionally, however, they enter prophase and the homologues may even pair and form chiasmata but their stainability is never appreciably improved (plate II, figs. 1-4). On the contrary in those cells in which only one or two fully condensed bivalents make their appearance, the remaining members of the complement also show a fair degree of condensation, their stainability is particularly improved and they do not present a woolly appearance so characteristic in the former case (plate II, figs. 6-12). These chromosomes in many cases, are able to pair and form chiasmata, although the latter formed bivalents never quite catch up with others in their degree of condensation. An intermediate behaviour with regard to their stainability and spiralisation is shown by the affected chromosomes in those cells in which three or four fully condensed bivalents are present (plate II, fig. 5).

Thus the degree of retarded chromosomes is related to and determined by the number of normal bivalents in each nucleus. Apart from this, the behaviour of the retarded chromosomes is more variable when only one or two normal bivalents are present. In such cells they may appear as retarded bivalents with regular or rather fuzzy outlines (plate II, *cf.* figs. 6 and 9). They may show failure of pairing (plate II, fig. 10), or they may be clumped, a condition with or without stickiness (plate II, *cf.* figs. 7 and 11).

It may also be emphasised that normal and retarded chromosomes are found to be randomly distributed in the nucleus. There is no suggestion that they occur in groups.

Finally it should be pointed out that not all pollen mother cells in the treated material include retarded chromosomes, but as many as 60 per cent. may be affected in particular anthers. The number varies considerably, both with different treatments and also within the same treatment. Only certain of the genetically different clones respond to the temperature treatment and in these only some flowers in some of the spikes.

(iii) *Nucleoli*

Abnormal behaviour of the chromosomes is not the only change induced in the nucleus. Other changes, which appear to be correlated with them, concern the nucleolus. The retardation of various chromosomes during meiotic prophase is always accompanied by abnormal nucleolar activity. The main irregularity is that no single nucleolus has usually been formed in the preceding resting stage. Instead, several nucleolar bodies are formed, usually as many as the number of normally condensed chromosomes with which they are found to be closely associated (plate II, figs. 1, 3, 5-12). Their number thus usually varies from 1-6, depending upon the number of normally condensed bivalents. Sometimes, however, more than one nucleolar body may be associated with such chromosomes or, alternatively, more than one bivalent may be associated with a single nucleolus. In still other cases, the nucleolar bodies may be found detached from the chromosomes. But their proximity to normal chromosomes and not to the retarded ones can always be observed and indicates association at earlier stages.

Thus the nucleolar bodies are found in association with the condensed chromosomes only. Chromosomes which show failure of condensation never have any nucleolar material attached to them. It is also very obvious that the presence or absence of nucleolar material on the chromosomes is not determined by their particular state of condensation. The association with some chromosomes and not with others can also be seen at earlier stages of division when all of them are present in the same relatively uncondensed state (plate I, fig. 3).

There seems little doubt that with the failure of the nucleolar organiser, individual chromosomes can have their own nucleolar material in many of the cells ; the nucleolar bodies thus formed are best described as *adventitious* (plate I, fig. 4). In many other cells, however, a number of chromosomes fail to have attached nucleoli and these are the ones which show failure of normal condensation.

The number of abnormal chromosomes varies greatly, as already pointed out, but cells with one or two normal bivalents occur with the greatest frequency. These frequencies suggest that the chromosomes differ in their capacity to form the nucleoli after the organiser has been inactivated. The bivalents, however, cannot be distinguished from one another and the suggestion therefore cannot be confirmed.

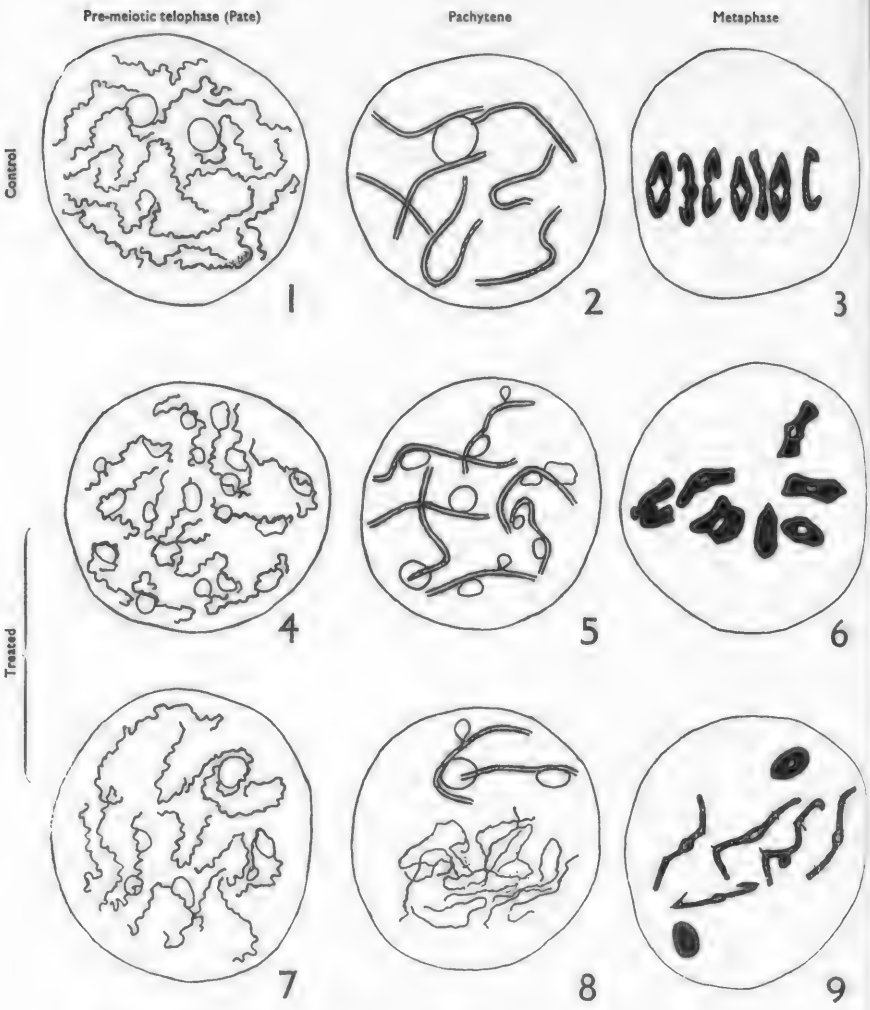


FIG. 1. Normal and abnormal nucleolar formation at pre-meiotic telophase and the subsequent behaviour of chromosomes in the control (figs. 1-3) and treated (figs. 4-9) material. 1. Normal formation of two nucleoli by the two organising chromosomes. 2. The seven bivalents synchronised in their development. 3. The normal metaphase arrangement. 4. Each of the fourteen chromosomes adventitiously forming nucleolar bodies. 5. The seven bivalents with associated nucleolar material and all synchronised as in 2. 6. Irregular metaphase arrangement; partial congression and failure of co-orientation. 7. Two pairs of chromosomes taking part in nucleolar synthesis. 8. Nucleolar-forming chromosomes showing pachytene pairing, others beginning to emerge from resting stage. 9. The two normal bivalents failing to congress, the retarded ones failing to co-orientate.

(iv) *Metaphase*

The non-synchronised development of chromosomes and the formation of adventitious nucleoli culminates in a metaphase condition which is highly irregular. The spindle in many of the PMC fails to be organised. In others the congression and co-orientation of bivalents on the metaphase plate are affected. When all the 7 bivalents in a cell are fully condensed, these may congress on the plate but their centromeres often fail to co-orientate showing instead an apparent lack of any orientation or a condition similar to auto-orientation of mitotic chromosomes. When both normal and retarded bivalents are present, the condensed ones may fail to show the metaphase arrangement and remain randomly distributed in the cell. The retarded ones, however, are often able to congress although their centromeres do not co-orientate.

The chromosomes following the irregular metaphase form one or more restitution nuclei, which apparently degenerate and no viable pollen grains are ever formed.

In the above account we have seen how the differential behaviour of chromosomes is to be associated with abnormal nucleolar activity. The diagram gives a general illustration of these and other results (fig. 1).

4. DISCUSSION AND CONCLUSIONS

For an understanding of the experimentally induced changes, two explanations are needed. First, the partial or complete failure of condensation in some members of the complement, when others condense quite normally, has to be explained. Secondly, it has to be determined whether this failure is in any way related to abnormal nucleolar formation, which is a characteristic feature of those nuclei whose chromosomes show such differences.

The suggestion that the irregular behaviour of chromosomes may be directly related to abnormal nucleolar formation is difficult to avoid. The nucleolar material is found to be associated with some of the chromosomes and not with others during earliest prophase, when all of them are present in a relatively uncondensed state. Similar differences in the association of the nucleolar material are still present during later stages of prophase, but the chromosomes are no longer found to be synchronised in their degree of condensation. Only the ones with nucleolar material attached appear normally condensed. The usual reduction in the size of the nucleolar bodies towards the end of prophase can also be seen. In view of these observations it is tempting to suppose that the nucleolar material may be responsible in some way for the condensation of chromosomes to which it is seen to be attached, while the lack of such association in others may account for their poor condensation. This is particularly so in view of the numerous reports in cytological literature which specifically attribute this role to the nucleolus substance.

Since nucleoli in most dividing cells disappear at the same time as chromosomes reach their maximum contraction and reappear in telophase with the reversal of prophase changes, many workers have assumed that the nucleolar material was incorporated into the chromosomes during their condensation. McClintock (1934) was one of the first to put forward this idea.

Darlington (1947) referred to the fact that when there is no organiser or nucleolus at all in an organism, there is always heterochromatin, and when there is no heterochromatin there is always a nucleolus. He took the broader view that the nucleolus and the charged heterochromatin evidently provide alternative or supplementary sources of the nucleic acid used in the charging of the prophase chromosomes. The possibility of the absorption of the nucleolar material into the substance of chromosomes has been suggested by White (1951) who pointed out that most authors have tended to regard the nucleoli as a temporary storage reservoir of material which is transferred to the chromosomes before metaphase.

If the suggestions made by McClintock and others are to be accepted, the induced differences in the condensation of different chromosomes in relation to nucleolar changes could have a simple explanation. With the inactivation of the nucleolar organiser as a result of temperature treatment, chromosomes synthesise individually the nucleolar material which remains associated with them, in the same way as in maize under conditions of a different nature. McClintock (1934) has shown that in pollen grains with defective nuclei—ones without the organising chromosome present—nucleolus-like droplets appear over the chromosomes generally. In others, particular genotypic changes may suppress the organising capacity of the nucleolar chromosome and induce the formation of adventitious nucleoli. However, many of the affected PMC in *Lolium* differ from the defective maize pollen grains in one important respect. In these following the inactivation of the organiser, not all members of the complement form the adventitious nucleoli. Some of the chromosomes fail to do so and these might be expected to remain uncondensed during prophase on the basis of McClintock's hypothesis. The dispersed nucleoli, unlike the normally organised ones, it might be held, do not contribute to the metabolism of all members of the complement but only of those to which they are attached.

A number of considerations, however, make it difficult to accept this simple explanation.

The concept of the transfer of nucleolar material to chromosomes and their consequent condensation, though very natural in view of their apparently related behaviour, is rendered unlikely in view of all the evidence. The Feulgen negative character of the nucleolus is well known and is readily demonstrated in the present material. Also the presence of ribose nucleic acid (RNA) in it has been demonstrated by a large number of workers. The discovery that RNA is

present in the chromosomes and more recently its reported increase in mitotic chromosomes compared with the interphase nucleus, may support the hypothesis of nucleolar contribution to the chromosomes. However, Jacobson and Webb (1952) who among others reported the increase, also pointed out that the ribonucleo-proteins appear on the chromosomes during the prophase while the nucleolus is still present and of normal size. In their view there is no evidence that the entire ribonucleo-protein of the chromosome is derived from the nucleolus. Moreover, the presence of RNA in the chromosomes has not obscured the fact that the nucleic acid content of the chromosomes is predominantly the Desoxyribose type. The possibility that the nucleolar RNA may be converted into Desoxyribose nucleic acid (DNA) of the chromosomes would be entirely opposed to the current views on the subject. Brachet (1947) who among others suggested that RNA may be a precursor of DNA, has since stated that the hypothesis of conversion seems doubtful. Mirsky (1951) is more definite and states that there is no evidence at present that DNA is formed from RNA or vice versa. The possibility that DNA may be controlling RNA synthesis has, however, been suggested by Rich and Watson (1954).

There are other good arguments against the views of McClintock and others. The quantitative instability of the ribonucleo-proteins of the nucleolus and of the cells in general, emphasised by several authors (Swift, 1953 ; Alfert, 1954 ; Mirsky, 1951) makes it difficult to relate the nucleolar material with a regular process like chromosome condensation. During the course of these and other cytological investigations on rye grass, not only great variations in nucleolar size have been observed, but it has been noted occasionally that metaphase chromosomes after the disappearance of nucleolar material may be condensed to a lesser degree than the diakinesis chromosomes in a neighbouring cell in which the nucleolus is still present. An alternative explanation for the disappearance of the nucleolus shortly before metaphase has been provided by the work of Caspersson and his collaborators (1950) who believe that the nucleolus is associated with cytoplasmic protein synthesis and its disappearance merely marks the end of that activity.

The above discussion shows that the induced differences in the degree of condensation of different chromosomes in the complement are not easily explained as due to the association of nucleolar material with a restricted number of chromosomes only. In view of this the first question may now be considered afresh in the light of other relevant evidence concerning chromosome condensation.

Corresponding with their cycle of spiralisation and despiralisation during mitosis, chromosomes are involved in another cycle of changes in their stainability and the amount of ultraviolet-absorbing materials concentrated on them. While the despiralised interphase chromosomes hardly stain at all, the maximally contracted metaphase chromosomes

do so very intensely. The prophase condensation of the chromosome threads has been attributed to the material, the so-called matrix which is responsible for their increased stainability (Wilson and Coleman, 1952 ; Cleveland, 1949). As for the nature of this substance, cytochemical methods and ultraviolet absorption techniques show that desoxyribo-nucleohistones are concerned (Jacobson and Webb, 1952*a* ; Alfert, 1954). Apart from the histones which Mirsky and Ris (1951) suppose to be quantitatively correlated with DNA, the protein content of nuclei decreases during division cycle. Discussing this, Pollister (1952) points out that the increase in DNA concentration as the chromatin condenses into compact chromosomes is not accompanied by corresponding protein condensation and that therefore the actual protein mass must be very much less in the metaphase chromosomes.

The evidence thus leaves little doubt that the prophase condensation of chromosomes is brought about by the concentration of the DNA-histones on the interphase threads. Darlington (1955) has described DNA as the essential agent in spiralisation and as evidence points to the fact that in certain polyploid plants and in the X-chromosome of hamster and other mammals, spiralisation fails with extreme starvation. As to the mechanism of the process Darlington has stressed the importance of residual protein. Referring to the work of Mirsky and Ris, he explains that it is the combination of polymerised DNA and residual protein which maintains the fibrous structure and thus must determine the spiralisation of chromosomes. Caspersson (1950) has suggested a specific mechanism for the process. According to him, the chromosome contracts with spiralisation: a phenomenon which might be caused by the small differences in the spacing of the nucleotides and polypeptide chains.

The failure of some of the chromosomes to condense must therefore be considered in terms of changes in their DNA content. The explanation needed is how some of the chromosomes come to have less than the normal amount of DNA that ensures complete spiralisation in others. The possibility that the failure of chromosome condensation may merely indicate a failure of the DNA to concentrate on them rather than its actual shortage has been considered. It does not, however, accord with the observed behaviour of the affected chromosomes. It has already been pointed out that the extent of failure of condensation in the retarded chromosomes is determined by the number of normal chromosomes present in the nucleus: the greater the number of such chromosomes, more complete is the failure of condensation in others. This relationship cannot be explained except on the basis of quantitative differences, which suggest competition between the normal and affected members of the complement.

The changes in the DNA content of different chromosomes evidently arise during interphase, for it has been noticed in many cases that the affected chromosomes barely emerge from that state (plate II, fig. 1). Moreover, this is also the stage according to recent

studies of mitosis if not of meiosis when synthesis of fresh DNA starts during the course of mitosis (Swift, 1953 ; Alfert, 1954 ; Walker and Yates, 1952). In meiosis synthetic activity it would appear is continued during prophase or, as Ansley (1954) has reported in the centipede, is confined to leptotene and zygotene.

In order to understand how these quantitative changes arise, it is necessary to refer to the distribution of DNA content of the interphase nucleus in relation to chromosome threads. Evidence on this point is not conclusive, and it is not definitely known whether the DNA leaves the chromosomes during this stage or merely becomes less concentrated.

If the DNA is detached from the chromosomes and dispersed in the nuclear sap, the possibility of a competition between various chromosomes towards the end of interphase is not difficult to visualise. Also if the synthesis of DNA has not been complete, some chromosomes may be more successful than others purely on a chance basis. The evidence, however, is strongly against this view. Since the normal and retarded chromosomes are found to be randomly distributed, differences in their degree of condensation cannot be explained by postulating the existence of a nucleoplasmic gradient in the nucleus. Moreover, there is evidence that successful condensation, though not a function of particular chromosomes, is determined by factors intrinsic to the chromosomes rather than by circumstances outside their control : the homologous chromosomes always respond to the temperature treatment in the same manner—both showing normal condensation or its failure. This would certainly not be the case if successful condensation or its failure is determined on a random basis.

A more generally accepted view about the state of chromosomes in the interphase nucleus is that the chromatin material does not leave the chromosome threads at all, it merely becomes loosely attached. The swollen DNA histone component of the chromosomes is then described as existing in an extended state (Ris and Mirsky, 1949 ; Pollister, 1952). Following fresh synthesis, DNA begins to concentrate on the chromosome threads once again, when they make their prophase appearance. On this view, shortage in the DNA content of a number of chromosomes will directly result, if they failed to take part in the synthesis of fresh DNA. In other words, the reduced DNA content of a number of chromosomes is simply accounted for by their reduced synthetic activity for this substance. This is an interesting possibility in view of the evidence that the normally and poorly condensing chromosomes are characterised by differing synthesising capacities. This refers, of course, to their synthesis of nucleolar material.

It has already been described that with the inactivation of the nucleolar organiser, not all the chromosomes in an affected cell may synthesise the nucleolar material. It may be that the chromosomes show similar differences with regard to DNA synthesis. The observation that only the nucleolus-forming chromosomes show normal

condensation may be interpreted as indicating that the capacities for nucleolar and DNA synthesis are correlated.

The reduced synthetic activity of the chromosomes may help to explain their failure of condensation. But the extent of this failure varies and is determined by the number of normally condensed chromosomes present in the nucleus. How do the normally condensed chromosomes exercise this control? It would appear that the chromosomes with reduced activity have to compete with the normal ones for a limited amount of precursor material. In that case, as the number of normals increases, the amount available to the affected ones will be proportionately decreased.

An attempt has been made in the above discussion to interpret the essential features of the observations described earlier. The suggestion regarding the correlated capacities for nucleolar and DNA synthesis, emerging from this, could be considered further if only briefly. It may be that the two syntheses in the chromosomes are under a common control. This finds support in the observations made by Caspersson (1950) and Schultz (1947), based on their cytological studies of salivary gland chromosomes. To quote Schultz's own words, " The same region which synthesises blocks of desoxynucleoproteins in the chromosome has to do with ribonucleo-protein synthesis in nucleolus and cytoplasm and with different protein component of the chromosomes ". This region in their view is the heterochromatin.

A number of considerations, however, indicate that the role of heterochromatin in this connection is not to be overstressed. Darlington (1947) has pointed out that many organisms lack both nucleolar organiser and heterochromatin, but the nucleoli are still formed. Moreover, when both are present, the organiser may lie in the heterochromatin or outside it. The observations of McClintock discussed earlier show that the function of heterochromatic nucleolar regions in maize is to organise material formed elsewhere in the chromosomes. In the absence of the organiser, the nucleolar material appears on all the chromosomes. That this is also what happens in rye grass when the organiser is inactivated has already been described in detail. These observations thus indicate that the role of euchromatic regions in synthesis cannot be ruled out.

5. SUMMARY

1. In certain clones of *Lolium perenne*, high temperature treatment at certain stages which include the telophase before meiosis, inactivates the nucleolar organiser in nearly all PMC. It disperses the materials so that nucleoli are formed by 1-7 bivalents ; the partner chromosomes always act in the same manner.

2. When less than 7 bivalents form nucleoli, the non-nucleolar chromosomes are retarded in their DNA-reproduction and prophase condensation. The fewer bivalents are retarded, the more they are

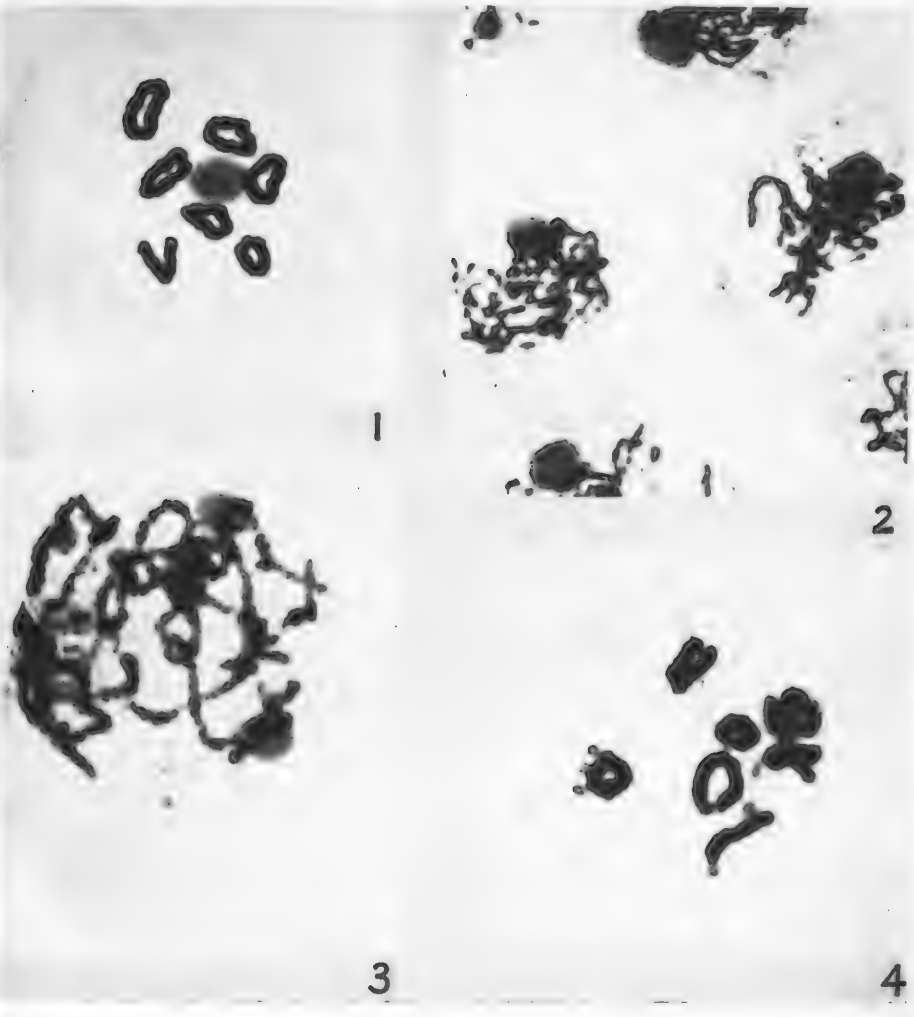


Plate I

FIG. 1.—PMC showing the uniform condensation of all the seven bivalents at diakinesis in the control material. A single nucleolus can also be seen. ×2075.

FIG. 2.—A patch of normal cells. A single nucleolus is seen in each of the prophase nuclei. ×1600.

FIG. 3.—An affected cell at early prophase showing the nucleolar material associated with some of the chromosomes and not with the others, before condensation has occurred. Eight nucleolar bodies differing in size can be recognised. 58 hours, 35±2° C. ×2075.

FIG. 4.—Diakinesis in an affected cell. Each of the seven bivalents has nucleolar bodies on it and all appear normally condensed. 38 hours, 35±2° C. ×2075.

Plate II

Diakinesis stages of the normal chromosomes in PMC showing the effects of treatment.

FIG. 1.—Six normally condensed bivalents and two remaining chromosomes which have hardly emerged from their interphase condition can be seen. The nucleolar material is seen on all the normally condensed chromosomes. 58 hours, 35±2° C. ×1925.

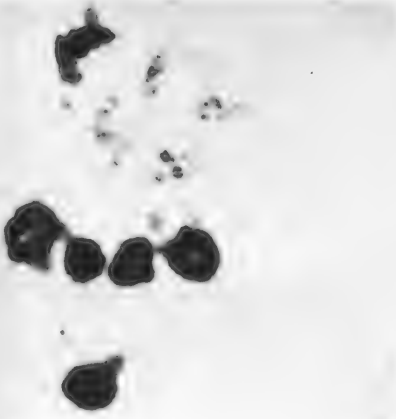
FIG. 2.—Five normal bivalents and two others which are underspiralised and show reduced stainability, can be seen. 58 hours, 35°±2° C. ×2525.

FIG. 5.—Three fully condensed bivalents each associated with a nucleolar body. The affected chromosomes showing failure of condensation and reduced stainability. 38 hours, 35±2° C. ×2525.

FIG. 6.—Two normally condensed bivalents, both attached to a single nucleolus. The remaining chromosomes correspond to a diplotene stage. Rod bivalent at 2 o'clock. 38 hours, 35±2° C. ×2075.

FIG. 9.—One normally condensed bivalent with a reduced nucleolus. The partially condensed chromosomes appear as in a diplotene nucleus. 58 hours, 35±2° C. ×1925.

FIG. 10.—One fully condensed bivalent with the nucleolus attached to it. The remaining chromosomes almost normally condensed but show failure of pairing. 38 hours, 35±2° C. ×2075.



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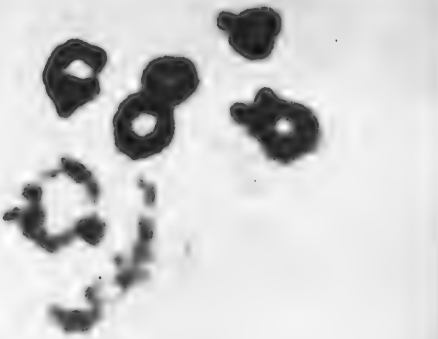

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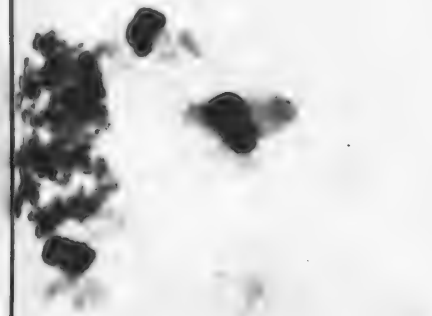

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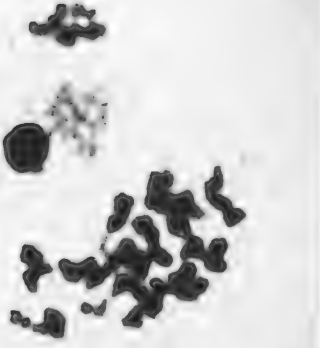

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Plate III

Diakinesis stages of the normal chromosomes in PMC showing the effects of treatment.

FIG. 3.—Six normally condensed bivalents having nucleolar material on them. The remaining two failing in their prophase development. 58 hours, 35±2° C. ×2525.

FIG. 4.—Five normally condensed bivalents and the affected chromosomes showing somewhat improved stainability. 58 hours, 35±2° C. ×1925.

FIG. 7.—Two fully condensed bivalents with prominent nucleolar bodies. The remaining chromosomes clumped and showing good stainability. 58 hours, 35±2° C. ×1925.

FIG. 8.—Two normally condensed bivalents, each with a nucleolar body. The affected chromosomes showing failure of normal condensation but their stainability is not much reduced. 58 hours, 35±2° C. ×1925.

FIG. 11.—One fully condensed bivalent with a large nucleolus attached to it. The remaining chromosomes showing stickiness. 14 hours, 35±2° C. ×2525.

FIG. 12.—One fully condensed bivalent with the nucleolus attached to it can be seen. The remaining chromosomes show good stainability and appear clumped. 14 hours, 35±2° C. ×1925.

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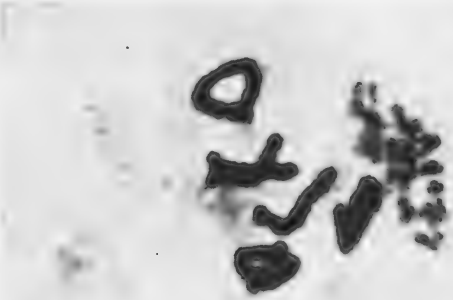

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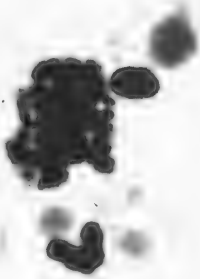

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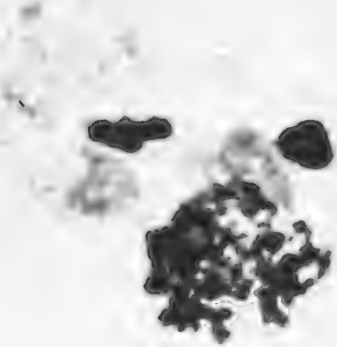

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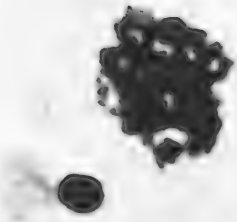

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retarded. Thus the normal and retarded chromosomes in a nucleus show a competition effect.

3. When all 7 bivalents form nucleoli they undergo normal condensation, and are able to organise a spindle but their centromeres fail to co-orientate. They show auto-orientation as in mitotic chromosomes or a lack of any definite orientation. In cells with mixed nuclei, the retarded bivalents also show failure of co-orientation but appear to be more efficient than the normal ones in their congression on the metaphase plate.

4. Thus dispersal of the nucleolus leads to two parallel systems of development in the same nucleus : nucleolar bivalents which are normal except for the centromere and non-nucleolar ones which are abnormal in their DNA-conditioned development.

5. Normal nucleoli might be regarded as available for metabolism of all chromosomes and dispersed nucleoli as available only for those to which they are attached. Or, perhaps preferably, chromosomes forming dispersed nucleoli might be capable of normal DNA-conditioned development while others are not. This leads to the suggestion that the capacities for nucleolar and DNA synthesis in a chromosome are correlated.

Acknowledgments.—I am greatly indebted to Prof. P. T. Thomas for suggesting the experiments and for advice and helpful criticism during the course of this work. My thanks are also due to Prof. E. T. Jones and Mr A. R. Beddows for providing the experimental material and for their kind encouragement. The award of one of their Science Research Scholarships by the Royal Commissioners for the Exhibition of 1851, which enabled me to undertake this work, is gratefully acknowledged.

Note.—The observations described and discussed in this paper were submitted to the University of Wales as part of a Ph.D. thesis.

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THE INFLUENCE OF TEMPERATURE ON MEIOSIS

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1. INTRODUCTION

THE main purpose of this investigation has been to study the influence of temperature on meiosis but the results have also been used to gain further knowledge of chromosome structure and behaviour. Many previous experiments have shown that changes in temperature can affect the general pattern of meiosis as well as the form and behaviour of the chromosomes (Plough, 1917, 1921 ; White, 1934 ; Barber, 1941, 1942 ; Swanson, 1941, 1942*a* and *b*, 1943, and Elliott, 1955). These earlier workers frequently considered only the influence of isolated temperatures or a comparatively small range of temperatures.

Chromosomes may exhibit three major effects from changes in temperature. These are alterations in the frequency and changes in the position of chiasmata and variations in internal coiling. All three effects may occur simultaneously but for convenience chromosome coiling will be considered separately. Other minor effects will also be considered.

2. MATERIALS AND METHODS

Clone 20[1] of *Tradescantia bracteata* and plants of *Uvularia perfoliata* have been used in these experiments.

T. bracteata ($2n = 12$) has six bivalents indistinguishable at meiosis. Tests with it were carried out from August to October. Plants of *T. bracteata* were grown for at least seven days in controlled temperature at 15° C. Some were then placed in cabinets each maintained at one of seven temperatures between 15·5° C. and 41·0° C.—the latter being the thermal death point of this plant. The cabinets were illuminated for 16 hours per day and the temperature controlled to within ±1° C. of the experimental treatment. Chiasma frequencies were determined in plants which had been given different lengths of treatment. Pollen mother cell squashes were made using acetic-orcein as stain fixative.

In *U. perfoliata* ($2n = 14$), meiosis takes place during March before the shoot appears above the ground. Control plants were grown at 6° C. Experimental plants were grown in incubators at various temperatures between 17° C. and 38° C. The thermal death point of this plant lies between 38°-41° C.

The seven bivalents of *U. perfoliata* are of three types as follows :—

Type A : 1 bivalent of long chromosomes with median centromeres. Chromosome length at metaphase of mitosis—18 μ.

Type B : 3 bivalents of long chromosomes with subterminal centromeres. Mean arm length—8·3 and 3 μ. Mean chromosome length—11·3 μ.

Type C : 3 bivalents of short chromosomes. Mean chromosome length—4·2 μ.

The chiasmata of the long and short arms of the *Type B* bivalents have been scored separately.

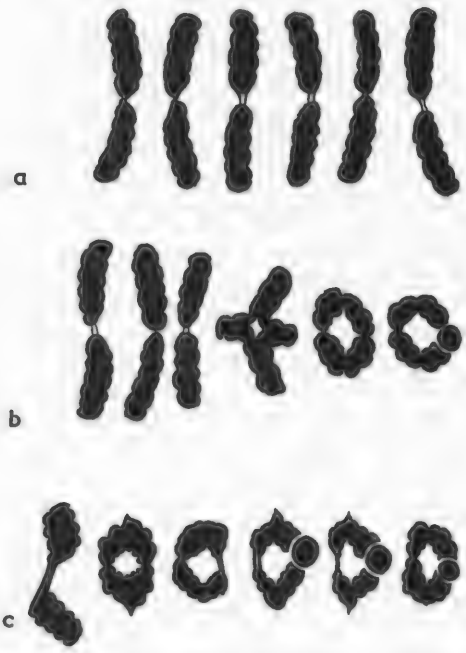


FIG. 1.—*T. bracteata*. The effect of time and temperature on material treated at 34° C. (*a*) control; (*b*) 12 hours at 34° C. and (*c*) 24 hours at 34° C. ×2000.

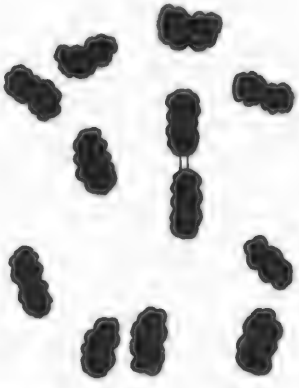


FIG. 2.—*T. bracteata*. Asynaptic MI cell with normal spiralisation after 48 hours at 39° C. ×2000.

Anthers were fixed in acetic alcohol and prepared as squashes after staining by the Feulgen reaction.

No recovery time was allowed between treatment and the time of scoring.

3. CHIASMA FREQUENCY AND POSITION

(*a*) Tradescantia bracteata

Under field conditions there is terminal localisation of the chiasmata, interstitials being usually less than 2 per cent. Between 15° C. and

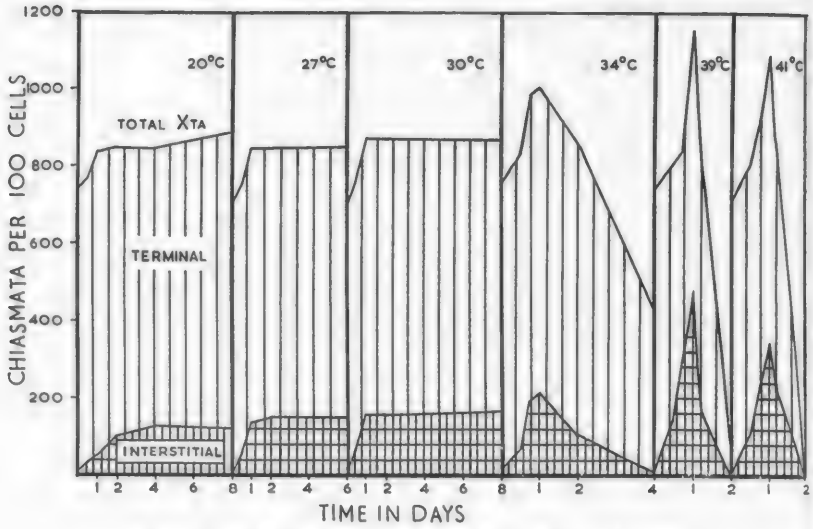
TABLE 1

The effect of six temperatures on chiasma frequency and position in samples of 100 PMC of T. bracteata *(controls at 15° C.)*

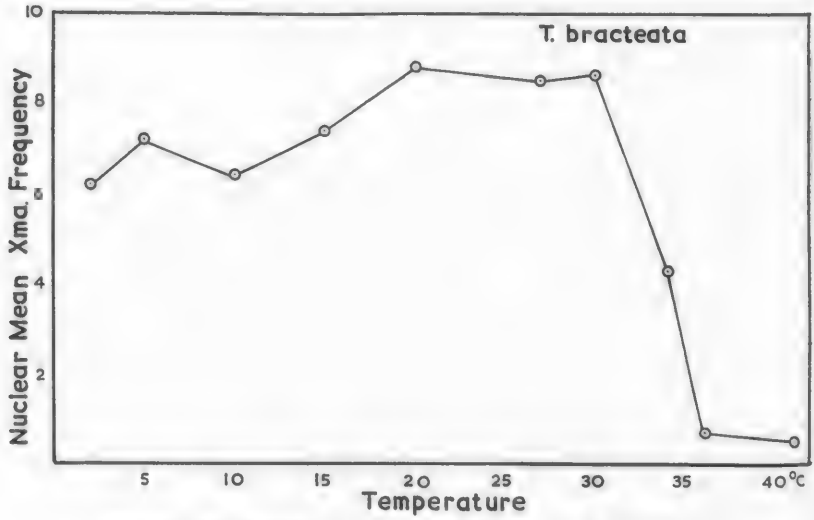
Temp.	Time in hr.	Interstitial chiasmata	Total chiasmata	Temp.	Time in hr.	Interstitial chiasmata	Total chiasmata
Control	...	17	743	Control	...	17	757
20·0° C.	*12*	37	773	34·0° C.	*12*	73	832
	24	52	836		*18*	186	984
	48	101	847		*24*	217	1006
	96	129	842		*48*	104	860
	192	121	883		*96*	3	431
Control	...	14	707	Control	...	17	743
27·0° C.	*12*	56	757	39·0° C.	*12*	81	810
	24	134	845		*18*	141	841
	48	148	849		*24*	475	1152
	144	147	851		*27*	283	961
					30	152	787
					48	0	72
Control	...	14	707	Control	...	14	707
30·0° C.	*12*	61	762	41·0° C.	*12*	110	805
	24	157	871		*18*	232	930
	48	157	868		*24*	339	1084
	192	162	870		*27*	226	868
					48	0	56

34° C. raising the temperature increases the number of interstitial chiasmata. This occurs gradually during the first 24 hours of treatment after which the number remains constant. After a slight rise at the 12 hour treatment, the frequency of terminal chiasmata also remains constant (table 1 and fig. 1). From the appearance of the bivalents at diakinesis and MI it seems likely that chiasmata can be formed closer to the centromere than is usual under field conditions.

Above 34° C. there is a further rise in the frequency of interstitial chiasmata during the initial period but the frequency rapidly falls off as the time of treatment increases—from 21 per cent. after 24 hours at 34° C. to less than one per cent. after 96 hours at the same temperature. The frequency of terminals is also lowered above 34° C. though more slowly than interstitial ones. As a result of loss of chiasmata of both types MI cells at temperatures above 34° C. are almost completely asynaptic (fig. 2). Such cells die. The highest



GRAPH 1.—The effect of temperature and time on chiasma frequency and position in *T. bracteata*.



GRAPH 2.—The relationship between chiasma frequency and temperature in *T. bracteata* after long periods of treatment.

total of chiasmata is seen after 24 hours at 39° C. when it approaches 1200 in 100 cells (graph 1).

According to Snoad (unpub.) lowering the temperature below 15° C. results first in a slight fall in chiasma frequency. A rise follows and then another fall affecting both terminal and interstitial chiasmata.

The influence of temperature after long periods of treatment is shown in graph 2.

(*b*) Uvularia perfoliata

The various forms of bivalents have already been described. Under control conditions there are approximately 20 chiasmata per cell, six or seven being usually terminal. Chiasma frequency is indirectly proportional to chromosome length, long arms have more chiasmata than short but have a lower proportion of terminals (graph 3, table 2).

As in *T. bracteata* chiasma frequency is correlated with temperature (graphs 3 and 4). Increases between 6° and 21° C. give more interstitial and, to a lesser extent, terminal chiasmata. Beyond 21° C. chiasma frequency falls owing to first, a reduction in interstitial chiasmata and also, at the highest temperatures, of terminals. The *Type A* bivalents and the long arms of the *Type B* bivalents in *U. perfoliata* have, however, a secondary region of chiasma formation (a secondary "contact point") close to the centromere. Consequently their terminalisation coefficient (T.C.) is at this stage lower than that of the other bivalents of the complement. After 24 hours' treatment at 38° C. there is complete asynapsis (fig. 3). In *U. perfoliata*, unlike *T. bracteata*, duration of treatment makes no difference to the effect.

The effects of temperature are most clearly seen in those chromosomes with most interstitial chiasmata. Arms of similar length behave similarly. Thus the long and short arms of *Type B* bivalents behave like *Type A* and *Type C* bivalents respectively.

As a result of the differential loss of chiasmata at the highest temperatures the chiasma frequency and distribution in the different bivalent types becomes similar as temperature is increased.

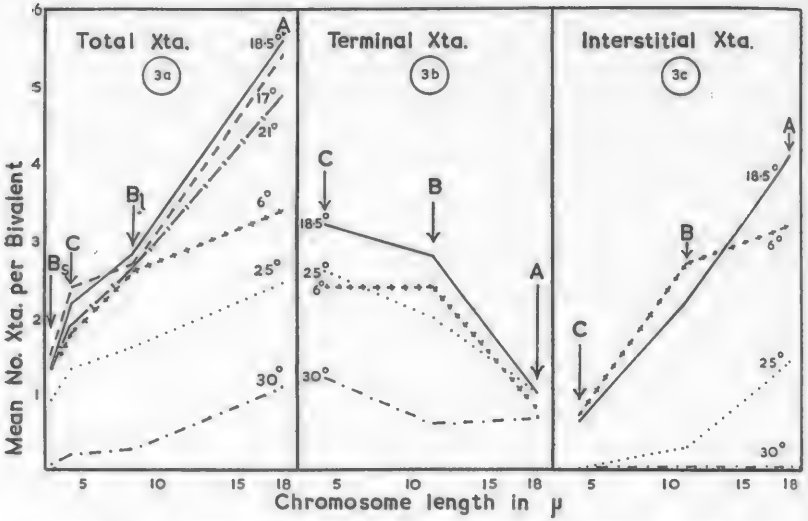
4. CHROMOSOME COILING

(*a*) Tradescantia bracteata

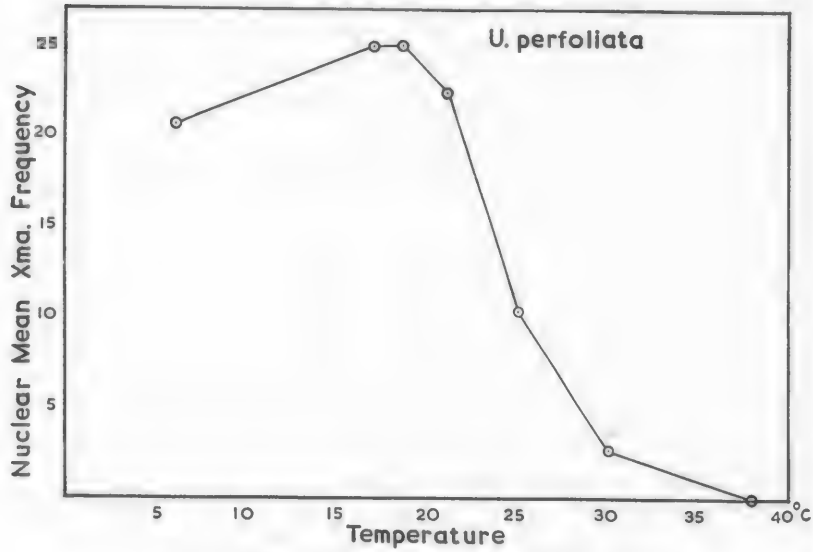
Below 34° C. coiling follows a normal course while at higher temperatures abnormalities are found. After 6-12 hours' treatment above 34° C. the ends of the free arms of some rod bivalents become despiralised—only a small part of a single major coil is involved, the remainder of the chromosome remains coiled normally (fig. 4).

After 12 hours more complete despiralisation of the major coils occurs. It begins at only three points. Most frequently it starts at a chiasma, less frequently at the centromere and only occasionally at the end of the free arm (table 3, fig. 4 and plate II, figs. 6-12).

Eventually the major coils disappear completely and the MI



GRAPH 3.—*U. perfoliata*. Chiasma frequency and position in relation to chromosome length at 7 different temperatures. The frequencies at 17·0° C., 18·5° C. and 21·0° C. are very similar and in Graphs 3*b* and 3*c* the frequencies at 17° C. and 21° C. have therefore been omitted. The keys to Graphs 3*b* and 3*c* are the same as that of Graph 3*a*. In Graph 3*a* the two arms of the *Type B* bivalents have been plotted separately.



GRAPH 4.—The relationship between chiasma frequency and temperature in *U. perfoliata*.

TABLE 2

The effect of temperature on chiasma frequency and position in samples of 50 PMC of U. perfoliata

Temp.	Type A bivalents (50)			Type B bivalents (150)									Type C bivalents (150)			All bivalents (50 cells)			
				Total		Term.		T.C.											
	Total	Term.	T.C.	L	S	L	S	L	S	Total	Term.	T.C.	Total	Term.	T.C.	Total	Term.	T.C.	Mean per cell
6° C.	171	20	*0·12*	397	190	99	83	*0·25*	*0·44*	587	182	*0·31*	279	175	*0·62*	1037	377	*0·36*	**20·7**
17° C.	1094	92	*0·08*	1636	838	356	326	*0·22*	*0·38*	2474	682	*0·28*	1408	941	*0·67*	4976	1715	*0·34*	**24·9**
18·5° C.	842	70	*0·09*	1233	669	306	233	*0·25*	*0·35*	1902	519	*0·27*	996	724	*0·72*	3739	1313	*0·35*	**24·9**
21° C.	487	54	*0·1*	796	384	169	153	*0·22*	*0·4*	1180	322	*0·28*	580	470	*0·81*	2247	846	*0·38*	**22·4**
25° C.	122	53	*0·5*	471	265	172	148	*0·37*	*0·56*	496	320	*0·66*	400	393	*0·98*	1018	766	*0·76*	**10·2**
30° C.	45	34	*0·8*	75	42	57	35	*0·76*	*0·84*	117	92	*0·78*	110	103	*0·9*	272	229	*0·83*	**2·7**
38° C.	0	0	...	0	0	0	0	...	...	0	0	...	0	0	...	0	0	...	**0**

The chiasmata of the long and short arms of the *Type B* bivalents have been scored separately and are shown under the columns headed L and S respectively. The *Terminalisation Coefficient* (T.C.) is calculated as the number of terminal chiasmata divided by the total number of chiasmata.

bivalents become long and thin (fig. 5, plate II, figs. 14 and 15). After 24 hours at 39° C. all but one per cent. of bivalents are of this type. Their appearance is reminiscent of *Triton* chromosomes. Both

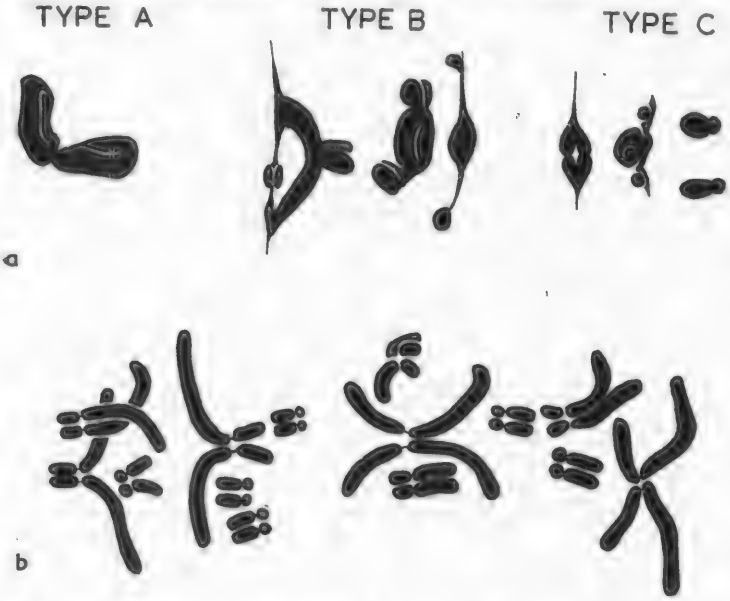


FIG. 3.—*U. perfoliata*. (*a*) MI after 6 days at 21° C., the three bivalent types have been separated; one *C* type is unpaired: (*b*) asynaptic MI devoid of major coils after 24 hours at 38° C. ×2000.

TABLE 3

The frequency of the different types of despiralisation found in T. bracteata *after 18 hours at 41° C.*

	Position of despiralisation		
	At chiasma	At centromere	At end of free arm
No. of bivalents . . .	183	36	5

chromosomes of one or two bivalents in 2 per cent. of these cells have additional, symmetrically arranged, constrictions. Most frequently they lie midway between the centromere and the end of the chromosome arm (fig. 5). Their constant size and position must result from the genetic property of the locus concerned, as in nucleic acid starvation (Darlington and La Cour, 1940).

After long treatments over 34° C. one per cent. of cells do, however,

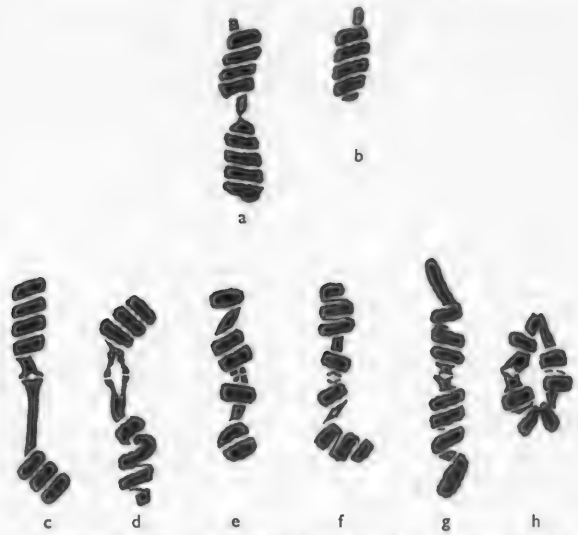


FIG. 4.—*T. bracteata.* (*a*) and (*b*) MI after 6 hours at 41° C. Uncoiling of a small part of a single major coil in (*a*) a bivalent and (*b*) a univalent. (*c*)-(*h*) bivalents from six different cells after 18 hours at 41° C. showing the various types of despiralisation.
(*c*) and (*d*) Uncoiling commencing at the chiasma.
(*e*) and (*f*) Uncoiling commencing at the centromere.
(*g*) Uncoiling commencing at the ends of the free arms.
(*h*) Uncoiling commencing at the chiasma and at the centromere. ×2200.

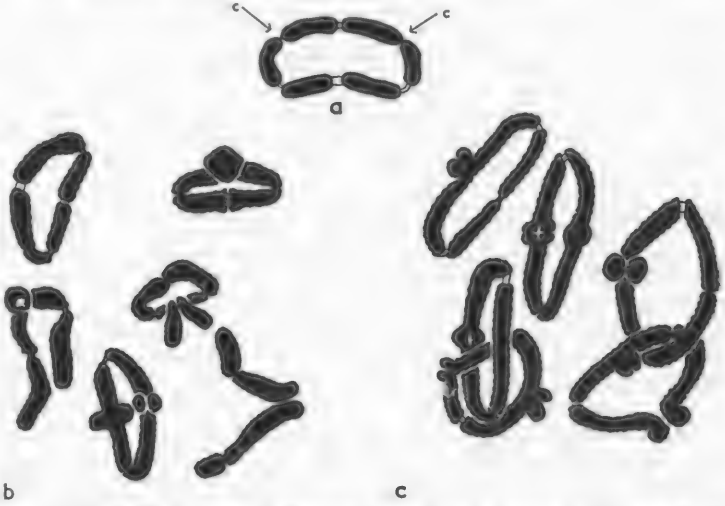


FIG. 5.—*T. bracteata.* (*a*) Single despiralised bivalent after 24 hours at 39° C. showing the additional constrictions, *c*, seen only in heat-treated material. (*b*) and (*c*) two cells showing the increase in chromosome length between (*b*) diakinesis and (*c*) MI after 24 hours at 41° C. Note the interlocking which occurs at high temperatures. ×2000.

show normal spiralisation but the chiasma frequency is reduced as mentioned previously (fig. 2).

One of the effects of heat treatment is to make the diffuse prophase of *T. bracteata* clearer and capable of analysis from diplotene onwards (plate II). After 24 hours' treatment at 39° C. bivalents at diplotene sometimes show incipient major coils which are never seen, however, in MI bivalents after the same and longer periods of treatment. Major coils can therefore be formed or begin to be formed only to disappear before metaphase.

Between diplotene and MI the bivalents elongate considerably but during this period there is no visible alteration in the coiling system (fig. 4). The increase in length probably results therefore from the relaxation of some internal, perhaps molecular, coiling. It is not known whether these chromosomes contain more material at MI than at diakinesis, but it may be significant that ribose nucleic acid and possibly other substances are incorporated in chromosomes towards the end of prophase (Kaufmann *et al.*, 1949 ; Jacobson and Webb, 1952 ; La Cour, 1954).

(*b*) Uvularia perfoliata

In *U. perfoliata* chromosome coiling at all temperatures below 25° C. is similar to that of material grown under field conditions. After 24 hours at 25° C. there is despiralisation of the major coils throughout the length of the chromosomes. Unlike *T. bracteata* this does not start at specific points but all parts of the chromosome are affected equally. After 24 hours at 30° C. many bivalents are devoid of major coils while after the same period at 38° C. there are no traces of major coils either at MI or during prophase.

5. DISCUSSION

The variations in chiasma frequencies brought about in *T. bracteata* and *U. perfoliata* by changes in temperature are similar to those found by Plough in *Drosophila* after consideration has been given to the mistake pointed out by Smith (1936). They are also similar to the results of Elliott with *Hyacinthus orientalis* and resemble those of White in species of *Locusta*, *Schistocerca* and *Stenobothrus*.

The reduction in chiasma frequency which occurs at high temperatures has not been demonstrated in animals nor in *H. orientalis*. While these temperatures would be lethal to the animals it seems likely that a reduction in chiasma frequency would have occurred in *H. orientalis* had the experiments been continued above 20° C.

Experiments involving change in chiasma frequency and position are best carried out in plants with localised chiasmata. Under field conditions about 98 per cent. of the chiasmata are formed terminally in *T. bracteata*. That they are not formed interstitially and subsequently terminalised is clear from the very small size of the fragments which occur after crossing over has taken place within inversions.

In *U. perfoliata*, on the other hand, the chiasmata are distributed along the length of the chromosome arms and up to eight may be found in the *Type A* bivalents. There is little or no terminalisation.

In the absence of movement the number and position of chiasmata are directly related to the degree of chromosome pairing at pachytene (Darlington, 1937). The differences in chiasma positions between *T. bracteata* and *U. perfoliata* must then be related to differences in the pairing behaviour of their chromosomes. In *T. bracteata* pairing sufficiently intimate for chiasma formation is largely confined to the chromosome ends. Increase in temperature changes this localisation. Between 15° and 34° C. either pairing must be continued towards the centromere or another region near the centromere must become paired. This will increase the probability of the formation of interstitial chiasmata. Pairing at the chromosome ends must be unaffected since chiasma frequency at these points is unaltered.

At all temperatures between 6° and 21° C. pairing of the *U. perfoliata* chromosomes is presumably complete and other factors which influence chiasma formation must be sought. Among possible causes may be mentioned the interference distance between chiasmata. It is well known that the formation of a chiasma reduces the probability of the occurrence of another in an adjacent region. The present experiments suggest that increases in temperature between 6° and 21° C. decrease the interference distances, possibly as a result of changes in the viscosity of the chromosomes.

The effect of high temperature is similar in the two species. There is a decrease in chiasma frequency from 34° to 41° C. in *T. bracteata* and from 21° to 38° C. in *U. perfoliata*. The decrease results from, first, a restriction of close pairing to the chromosome ends so that interstitial chiasmata are less frequently formed. Eventually there is an absence of pairing in both species and asynapsis results.

The maximum number of chiasmata are evidently formed at different temperatures in different species. This maximum is no doubt related to the conditions under which growth normally occurs. Most species will be adapted to give an optimum amount of crossing over at normal temperatures. The present experiments with *Tradescantia* and *Uvularia* suggest that this optimum is less than the maximum possible.

To explain the various changes in chiasma frequency, White suggested that changes in temperature bring about physical changes within the chromosomes and that the interference distance between chiasmata must be altered. The differential distance (Mather, 1940) would presumably be similarly affected. While decrease in interference distances will account for the increase in chiasma frequency in *U. perfoliata* it cannot be considered in relation to *T. bracteata*.

All cell processes will be affected by changes in temperature and these may lead directly or indirectly to changes in the number and position of the chiasmata. As a result of increase in temperature the

viscosity of the nuclear sap will probably be reduced. It appears that chromosome movement is speeded up more than the prophase as a whole. The increased chromosome movement might account for the high frequency of interlocked bivalents found in heat-treated material. At higher temperatures pairing, if it occurs at all, is confined to the chromosome ends and also in *U. perfoliata* to the centromere region of the long arms of *Type A* and *B* chromosomes. These are what Darlington (1940) calls the contact points in chromosome pairing.

The chromosomes undergo during the nuclear cycle a continuous and normally irreversible reduction in the number of coils. In these experiments, however, the reduction is reversed. As a result of spiralisation the chromosomes normally become progressively shorter. Swanson considers that low temperatures reduce the number of coils while high temperatures may completely inhibit their development. Observations in the present cases agree with these findings except that high temperatures do not necessarily completely inhibit the development of the major coils. They begin to form in *T. bracteata* and subsequently disappear.

The coiling spiralisation which occurs during normal prophase and which Swanson considered as uncoiling must not be confused with the abnormal despiralisation occurring at high temperatures. Coiling spiralisation, affecting all parts of the chromosome equally, decreases chromosome length, while the abnormal despiralisation, starting at specific points on the chromosome and extending along it, increases its length.

A comparison of meiotic chromosomes without major spirals with normal mitotic chromosomes is also of interest. As Darlington points out, the nucleolar constrictions are probably constrictions of the minor coils because they are not visible at MI. The small terminal nucleolar organisers of *T. bracteata*, visible at mitosis, are, however, not seen in the abnormal meiotic chromosomes which are devoid of major coils but have minor coils.

Similarly, in the abnormal meiotic chromosomes additional constrictions, constant in size and position, are seen. It is known that high as well as low temperatures lead to abnormality in heterochromatin (La Cour, 1954). *Tradescantia* cannot be grown satisfactorily at temperatures low enough for the study of heterochromatin. The abnormal constrictions seen in these *T. bracteata* bivalents at high temperature may then be heterochromatic and invisible at metaphase except at these temperatures of 34° C. or above. La Cour (unpub.) has recently found small segments of heterochromatin present in the resting nuclei of *T. bracteata*.

Any alteration in the number and position of the chiasmata would have evolutionary significance to the species concerned. A sudden rise in temperature in *T. bracteata* and *U. perfoliata* would release new genetic variability. Alteration of other agencies which affect chromosome movement, *e.g.* the amount of infra-red radiation, would probably

Plate I

The influence of temperature on chiasma frequency and position.

Tradescantia bracteata

FIG. 1.—MI from control material. ×1600.

FIG. 2.—MI after 18 hours at 34° C. ×1600.

FIG. 3.—Asynaptic MI after 24 hours at 39° C. ×1600.

Uvularia perfoliata

FIG. 4.—MI after 6 days at 21° C. : $6^{II}+2^{I}$. ×1000.

FIG. 5.—Asynaptic MI after 24 hours at 38° C. ×1000.

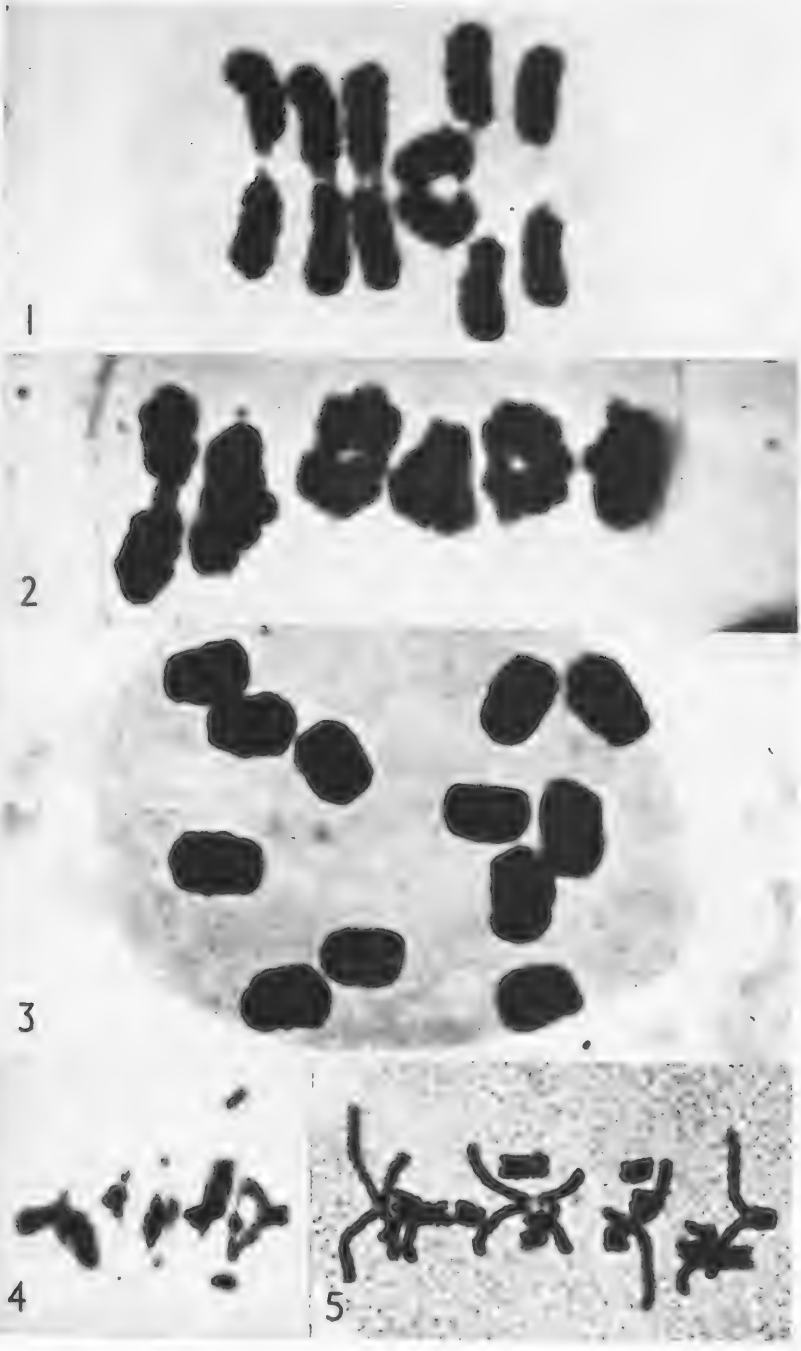

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Plate II

Tradescantia bracteata

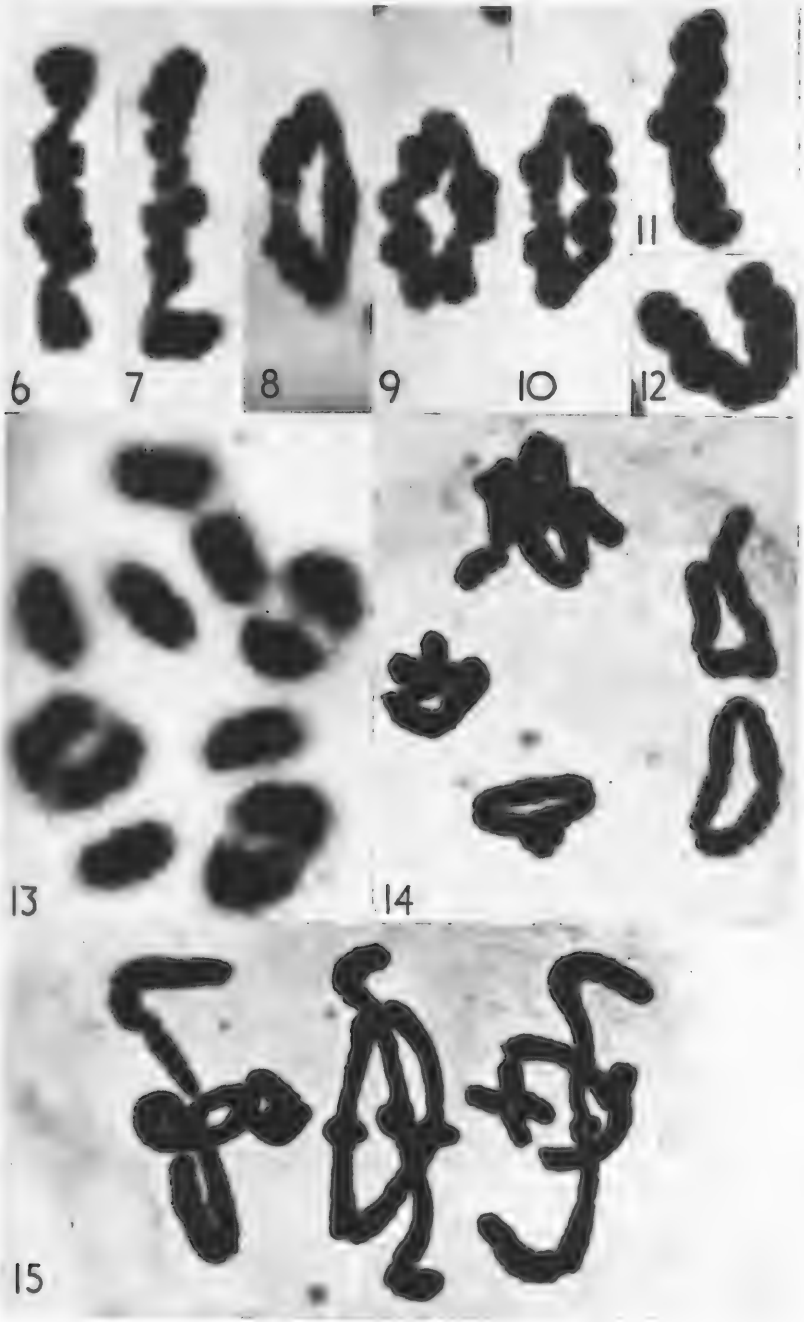
FIGS. 6-10.—Individual bivalents at MI after 18 hours at 41° C. showing the various types of despiralisation. ×2100.

FIGS. 11-12.—Individual bivalents at diakinesis after 24 hours at 39° C. Major coils can still be seen. ×2100.

FIG. 13.—Diplotene after 24 hours at 39° C. with partially developed major coils. These disappear by MI. ×2100.

FIG. 14.—Early MI after 24 hours at 41° C. (same as text fig. 5*b*). ×1600.

FIG. 15.—MI after 24 hours at 41° C. The ring bivalent at the right with two interstitial chiasmata has been broken by squashing at a centromere. The ring bivalent at the left shows relational coiling. ×1600.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have similar effects and treatments of this type may have considerable practical application in plant breeding.

6. SUMMARY

1. In *Tradescantia bracteata* and *Uvularia perfoliata* high temperatures affect (*a*) the number and position of the chiasmata, and (*b*) the coiling of the chromosomes at meiosis.

2. Increase in temperature results in an increase in the frequency of interstitial chiasmata. This, as Darlington (1940) has suggested, could be related to an increase in the rate of zygotene pairing. The increase is followed at higher temperatures by a fall in both terminal and interstitial chiasmata : asynapsis results.

3. At high temperatures prophase is much clearer than is usual in *T. bracteata*. Spiralisation is also abnormal : major coils open out and finally disappear. In *T. bracteata* as opposed to *U. perfoliata* the major coils begin to open out at specific points.

4. When major coils are absent constrictions, not normally visible, can be seen on some chromosomes of *T. bracteata*. These are probably in heterochromatic segments.

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FURTHER STUDIES ON ISOLATION IN THE BUTTERFLY *MANIOLA JURTINA* L.

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Our previous studies (W. H. D. and E. B. F., 1949-55) of the butterfly *Maniola jurtina* in the Isles of Scilly and elsewhere have established that the distribution of spots on the under-side of the hind-wings provides an effective method of quantifying variation in both sexes, and that it can be used as a means of detecting evolutionary divergence in different colonies. It would appear unlikely that the presence or absence of spots could, in itself, be of any selective value, and we must conclude that variations in spotting are associated with other characteristics, probably of a physiological kind.

One of the features of our work on *M. jurtina* in Scilly has been the discovery that, while the spot-distribution in the males is everywhere very similar, that of the females varies from one isolated colony to another but is stabilised at a constant value in each. One of our objects in August 1954 and 1955 was to study the effects of various types of ecological barriers in bringing about this isolation. This necessitated :

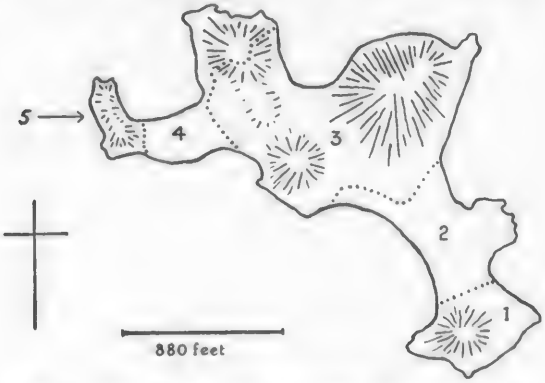
(*a*) confirmation of some of our findings in former years.
(*b*) an examination of certain localities which we had not previously visited.

I. SMALL ISLANDS

(*a*) *Tean* (see Map 1)

The conditions on the island in 1954 were much the same as they had been the year before. The growth of grass in area 2 (due to lack of grazing) and the inroads of bracken at its southern end had resulted in its union with area 1 as a single continuous locality. The grass in area 4 which, like area 2, had previously been short and lawn-like, was now long and had ceased to form a barrier to the movement of *M. jurtina*. Thus the island now contained two colonised localities, one at the southern extremity (areas 1 and 2), the other on the western promontory (areas 4 and 5). These were separated by area 3 which, until 1951, had supported a large population of *M. jurtina*. But the removal, in the late summer of 1950, of a herd of cattle had resulted in a heavy growth of the vegetation previously

controlled by grazing and trampling. By 1954, therefore, an impenetrable mass of bramble and bracken covered the whole of area 3. *M. jurtina* was now absent there, and the region thus constituted an effective barrier between the southern and western communities. On visiting the island in 1955 this situation was found to persist.



Map. 1. Tean (Isles of Scilly). Boundaries of collecting areas.

(i) *Males.* Our sampling in 1953 showed that the population of area 1 had maintained a stable spot-distribution since 1946, with a single large mode at two spots (4). The 1954 sample conformed closely with that of the previous year (table 1). The male spot-distribution in this colony had thus remained unchanged for a period

TABLE 1

Spot-distribution in male Maniola jurtina *on Tean (area 1) in 1953 and 1954*

Spots :	0	1	2	3	4	5	Total	Difference
1953 . . .	2	8	73	21	9	1	*114*	N.S.
1954 . . .	2	16	90	26	9	2	*145*	

In this and all subsequent tables, N.S. denotes "Not significant" and S. denotes "Significant". Figures for χ^2 are included where there is any question of doubt.

of nine years. It is typical of the Scillies in general, and also of southern England.

(ii) *Females.* Samples from areas 1 and 5 in 1954 are compared with those of the previous year in table 2. In area 1 spotting of the females, like that of the males, had remained unchanged, showing the bimodal distribution characteristic of the previous eight years with the smaller mode at no spots and the larger at two.

Our studies in 1953 had shown the colony in area 1 to be similar in spot-distribution to the small population in area 5. It was, therefore, of much interest to discover that in 1954 a significant change had taken place in the area 5 colony since the previous year (see table 2), and that its spot-distribution now differed once more from that in area 1 (see tables 3 and 4).

A significant difference between the two colonies had, in fact, become evident in 1951 when area 4 was still heavily grazed and constituted an ecological barrier, isolating area 5 from the rest of the island (3). It was, however, relatively slight, though the larger mode was then at 0 spots. This distinction had disappeared in 1953 when the area 5 and area 1 colonies were once more substantially the same (4), as in 1950.

TABLE 2

Spot-distribution in female Maniola jurtina *from two colonies on Tean in 1953 and 1954*

Spots :	0	1	2	3	4	5	Total	Difference
Area 1 (1953) .	32	11	46	21	3	1	*114*	N.S.
Area 1 (1954) .	30	14	49	11	1	1	*106*	
Area 5 (1953) .	30	14	54	13	3	0	*114*	$\chi^2_{(3)} = 8{\cdot}8$;
Area 5 (1954) .	13	18	68	11	5	0	*115*	$P < 0{\cdot}05$

We are thus faced with an interesting situation. Two populations have each expanded into a new area which was formerly a barrier to them. That in area 1 has extended across area 2, while that in area 5 has colonised area 4. The two colonies are now held apart by area 3 which has become a barrier. In so expanding, the population of area 1 (now that of areas 1 and 2) has remained unaltered, while the area 5 community (now that of areas 4 and 5) has undergone a change of spotting. Why has an extension of range had no effect in one locality but a marked effect in the other? A possible explanation is as follows. The population of area 1 has spread across area 2 without adjusting its spot-frequency because the ecology of the two places has become very similar. Not only has the grass ceased to be lawn-like, but bracken has encroached across most of area 2 thus affording much the same degree of shelter that has always been available to *M. jurtina* in area 1.

On the contrary, the population in area 5 has spread eastwards because area 4 has become habitable to it, although it is not at all like area 5. Colonisation has only been possible because of the growth of long grass. The sheltering bracken so characteristic of areas 1, 2 and 5 is absent from area 4, and the ecological conditions there are thus unlike those occurring anywhere else on the island.

It may, thus, be something more than a coincidence that in area 2, where a mixture of long grass and bracken prevails, the Tean population has retained its familiar characteristics; while, on the other hand, in area 4, which is quite different from any which has

TABLE 3

Spot-distribution in female Maniola jurtina *from different areas on Tean in 1954*

Spot numbers	Area 1	Area 2 (north edge)	Area 4	Area 5
0	30	31	13	13
1	14	19	17	18
2	49	55	62	68
3	11	3	8	11
4	1	0	6	5
5	1	0	3	0
Totals . .	*106*	*108*	*109*	*115*

previously supported *jurtina* on the island, the spotting has undergone a striking modification. This, of course, also affects the population in area 5 which is in no way isolated from that in area 4. Thus the single population now occupying both these areas has had to adjust

TABLE 4

Comparison of spotting in female Maniola jurtina *from different areas on Tean in 1954*

Areas	1	2*	4	5
1	...	N.S.	just >0.02	<0.02
2*	...	...	<0.001	<0.001
4	...	...	...	N.S.
5	...	...	...	...

* North edge.

Figures are values of P derived from $2 \times n$ tables. Comparisons are made by reading the figures or letters at the point where horizontal and vertical columns intersect.

itself to the average of the dissimilar ecological conditions which they provide, resulting in a different spot-distribution from any previously recorded on Tean.

Owing to other commitments it was only possible in 1955 to obtain a small sample of females from area 1 (table 5). The distribution of spotting is at least strongly suggestive that no change had taken place since the previous year.

(*b*) *Great Ganilly (males and females)*

In 1955, much of our work was centred on the eastern islands of Scilly (see map 2). It was thus possible to devote a day to collecting on Great Ganilly so as to obtain a sample of *jurtina* for comparison with those of 1951 and 1953 (3, 4).

TABLE 5

Spot-distribution in female Maniola jurtina *from Tean area 1 in 1955*

Spot numbers	Total
0	6
1	3
2	12
3	3
4	2
5	...
	26

TABLE 6

Spot-distribution in male Maniola jurtina *from Great Ganilly in 1951 and 1955*

Spots :	0	1	2	3	4	5	Total
1951 . .	0	2	33	27	13	1	*76*
1955 . .	0	1	20	10	1	2	*34*

TABLE 7

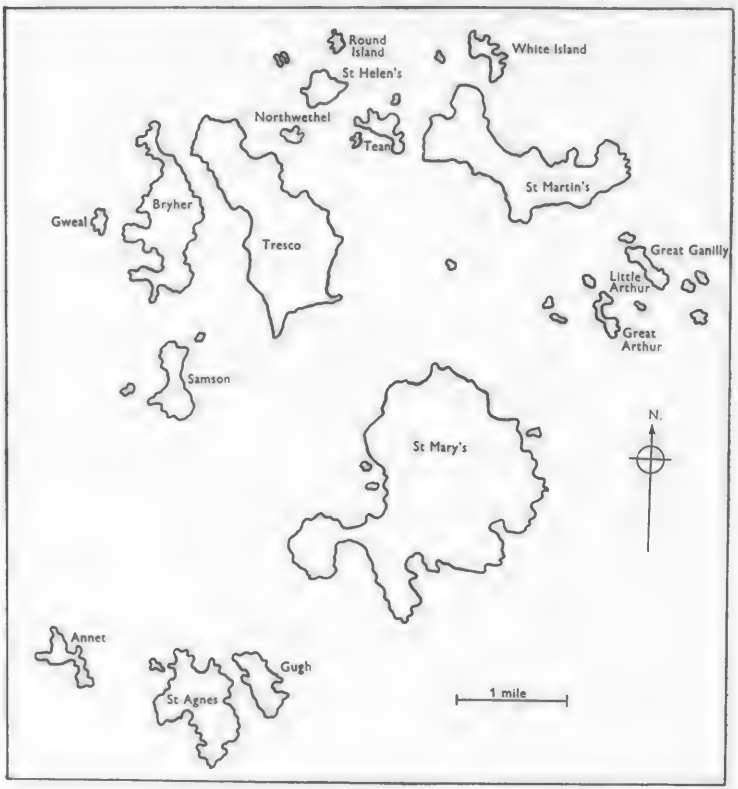
Spot-distribution in female Maniola jurtina *from Great Ganilly in 1951, 1953 and 1955*

Spots :	0	1	2	3	4	5	Total	Difference
1951	41	7	37	8	5	0	*98*	N.S. ($\chi^2_{(3)} = 6{\cdot}39$ P = 0·05 to 0·1)
1953	32	13	52	6	2	0	*105*	
1955	13	9	33	6	1	0	*62*	N.S. ($\chi^2_{(3)} = 2{\cdot}13$; P = 0·5 to 0·7)

The males, which emerge before the females, were somewhat scarce owing to the earliness of the season, but from a comparison of the small 1955 sample with that of 1951 (no male sample was taken in 1953) it is obvious that no significant change in spotting had occurred (see table 6).

The female sample was a larger one and is compared with those of 1951 and 1953 in table 7.

A comparison of the samples in 1951 and 1953, also of those in 1953 and 1955, shows no significant deviation in spotting. Nor are the data for the three seasons, considered together, heterogeneous ($\chi^2_{(6)} = 11{\cdot}5$, for which $P = 0{\cdot}05$ to $0{\cdot}1$). If, however, the 1951 and 1955 samples are compared, the value for $\chi^2_{(3)} = 9{\cdot}16$, for which P is between 0·05 and 0·02. There is thus good evidence of a change in



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Map. 2.

spot-distribution of female *M. jurtina* over a period of four years. This appears to be a consistent trend in the direction of reducing the mode at 0 spots and increasing that at 2 ; for though the 1953 sample does not differ significantly from the other two, it is intermediate between them in this respect. This is the first instance of its kind which we have encountered on a small island with a single, continuous population. The reasons for the change are, as yet, unknown, but they are no doubt ecological. The vegetation on Great Ganilly differs some-

what from that of any other small island we have studied so far, consisting of a luxuriant growth of various grasses. There is, in addition, much heather interspersed with short grass, which is to be found mainly on the higher ground. It may be that in these rather abnormal circumstances the insect is particularly sensitive to small environmental changes, but only further study can provide the data necessary for a more critical analysis of this situation.

(c) *White Island (females only)*

This is connected with St Martin's at low water by a tumble of seaweed covered rocks 225 yards long, exposed at about half tide. Probably, therefore, the population of *M. jurtina* inhabiting it is quite isolated, for we have found elsewhere that 100 to 150 yards of uninhabitable country is almost a complete barrier to this butterfly. Moreover, the distance involved is even greater than here indicated, for the insect is rare or absent at the end of White Island nearest to St Martin's, where the terrain, consisting of very short grass, rocks and some bracken, is unsuited to it.

White Island has an area of about 40 acres, so that it falls within the small-island group of Scilly. We collected on it in 1953, 1954 and 1955, and obtained the results shown in table 12. They are very consistent with one another : $\chi^2_{(4)} = 2{\cdot}6$, for which $P = 0{\cdot}7$ to $0{\cdot}5$. The population resembles rather closely the one which is isolated at the southern end of Bryher (4), in having a single mode at 0 spots ; also that occupying the mainland of southern England excluding Cornwall, though there the mode is much more pronounced (2, 3). It differs markedly from that found in the main (central) area of St Martin's (see tables 9 and 11).

When we proceed to a formal comparison between the two populations, we find that the difference at 0 spots contributes a χ^2 of no less than 19. At one spot, the proportions in each population are nearly the same and the contribution is only 0·3, while at 2 spots it is 9 and at 3 and more it is 19 owing to the almost complete absence of that condition on White Island. Thus the distinction between them proves to be very great. The problem raised by the similar spot-distributions of the *M. jurtina* population inhabiting White Island and that found on a small coastal strip of St Martin's immediately opposite is discussed on pp. 63 and 64.

2. THE LARGE ISLANDS

(a) *Tresco (females only)*

We had already sampled the *M. jurtina* population on Tresco in 1950 and 1951, and we did so again both in 1954 and 1955. The island may be said to fall into four main ecological divisions : the barren areas of rocky coast and poor heather country to the north, the great sub-tropical gardens with their sheltering pine woods, now

maintained so successfully by the Dorrien-Smith family for over a century, the uncultivated coast region of marram grass and short dune-turf upon sand, and the agricultural lands. *M. jurtina* is to be found only in the last two of these, and on the agricultural land only on rough grass and along hedgerows. It occurs also in a few small isolated areas. The population inhabiting one of these is described and discussed on pp. 61 and 62.

Both in 1954 and 1955 we collected over the extensive region where we had taken our previous samples. This we describe as the "Main Area". The insect was not particularly common in either year and we had some difficulty in catching sufficient numbers. Our results, and those obtained formerly, are given in table 9. An inspection of this table will indicate the close similarity of spot-distribution in this Main Area for all four years. This can be assessed from a table of General Contingency, in which the samples having three or more spots are accumulated to avoid unduly small or missing values. The association is measured by $\chi^2_{(9)} = 4{\cdot}8$, for which $P = 0{\cdot}9$ to $0{\cdot}8$. It will be appreciated, therefore, that the results are very concordant, and give no indication that the spot-distribution has changed during the period 1950 to 1955 inclusive.

(*b*) *St Mary's (females only)*

A very considerable part of this, the largest of the Isles, provides a suitable habitat for *M. jurtina*, which is widely distributed over it, being found along hedges, on rough ground and in pastures. As we had only collected on St Mary's once previously, in 1951, it was a matter of much interest to do so again in 1954 and 1955, and to determine whether the spot-distribution had remained constant over the whole period for which we now have data, as well as during the two intervening years for which no information is available. Our results for the three seasons in question are given in table 9. The agreement between them is measured by $\chi^2_{(6)} = 5{\cdot}9$, for which $P = 0{\cdot}5$ to $0{\cdot}3$.* Consequently they must be judged substantially similar.

(*c*) *St Martin's*

(i) *Females*. *M. jurtina* occurs commonly over a large area of St Martin's. It is to be found in regions of turf with marram grass along the coast, on rough ground, in pastures and by hedge sides. However, it is, as elsewhere, absent from heather moors and extensive areas of bracken. Such places, therefore, isolate a few small colonies here and there along the shore (see pp. 63-64) from the main population of the island. The latter has already been studied in 1950, 1951 and 1953, and we obtained further samples of it in 1954 and 1955. These data are given in table 9. The spot-distributions are in substantial

* Individuals with three or more spots were combined to avoid very small numbers.

agreement in all five years, their homogeneity over the whole period being measured by $\chi^2_{(12)} = 11{\cdot}6$, for which P is just below 0·5.

(ii) *Males*. We have tested the distribution of spotting in the males of *M. jurtina* extensively in the past. It has proved to be very similar not only on all the islands of Scilly, large and small, but throughout southern England, including Cornwall (2, 3). In view of shortage of time due to other and more pressing aspects of the work, we obtained only one male sample on the large islands during the two years now under review. This was collected on St Martin's in 1955. It is strictly in accord with the established form of male spotting, having a single large mode at 2 spots. In table 8 this 1955 sample is compared with those taken there in three previous years. The over-all agreement is measured by $\chi^2_{(9)} = 9{\cdot}6$, for which P = 0·5 to 0·3.

TABLE 8

Spot-distributions of male Maniola jurtina *from the Main Area of St Martin's*

Spots :	0	1	2	3	4	5	Total
1950 . .	5	16	66	20	7	3	*117*
1951 . .	8	18	199	43	18	7	*293*
1953 . .	...	7	37	9	3	...	*56*
1955 . .	1	9	49	15	7	...	*81*
	14	*50*	*351*	*87*	*35*	*10*	547

(d) *General comparison of female spotting on the large islands*

We have already pointed out in a previous report (4) that those islands in the Scilly archipelago on which we have studied *Maniola jurtina* fall into three size-groups : (*a*) large islands of 682 acres or more, (*b*) small islands of 40 acres or less, and (*c*) two, Bryher and Samson, of intermediate size. Thus the large and small islands are of very distinct areas : the difference being seventeen times or more. The spot-distributions of the *M. jurtina* populations differ greatly from one to another of the small islands on each of which, however, they have remained constant as long as we have investigated them (4, and pp. 51-57 of this paper). To this statement there are two exceptions : the marked and rapid change in one of the Tean populations associated with a complete alteration in the ecology of the area (p. 53), and the slow trend in spot-adjustment detected on Great Ganilly (p. 55). We have also shown (4) that neither Bryher nor Samson is inhabited by an extensive population, as are the large islands, but that there are two, or possibly more, restricted and isolated colonies on each. These, therefore, have the characteristics of those found on small islands.

As already explained, the spot-distributions on each of the three large islands have remained constant throughout our period of study. Moreover, they are of similar form on all of them, being approximately flat topped with high frequencies at 0, 1 and 2 spots which do not differ significantly from one another.

It was essential to determine if the constancy and similarity of spotting on the three large islands, revealed by our previous studies (3), is confirmed by the data obtained in 1954 and 1955. For the existence in *M. jurtina* of distinct types of population on the small and the large islands respectively is of much importance from an evolutionary point of view (3).

Our total results on all the large islands we have studied are shown

TABLE 9

Spot-distributions of female Maniola jurtina *on three large islands in Scilly*

Spots	Tresco				St Mary's			St Martin's					Total
	1950	1951	1954	1955	1951	1954	1955	1950	1951	1953	1954	1955	
0	43	39	26	32	36	26	47	33	38	46	76	79	*521*
1	37	40	28	31	27	26	37	35	38	34	72	72	*477*
2	41	47	36	27	31	31	46	32	33	36	78	68	*506*
3	7	7	5	7	4	13	13	7	5	3	8	18	*97*
4	2	1	1	4	3	4	7	2	2	1	3	7	*37*
5	...	1	...	...	...	...	1	...	...	...	...	...	*2*
	130	*135*	*96*	*101*	*101*	*100*	*151*	*109*	*116*	*120*	*237*	*244*	1640

in table 9. Their homogeneity is measured by $\chi^2_{(33)} = 36{\cdot}9$.* For this, the expression $\sqrt{2\chi^2} - \sqrt{2n-1}$ supplies a normal deviate. Here $\sqrt{73{\cdot}8} - \sqrt{65} = 0{\cdot}53$. Thus the data for all the available years on all three large islands are effectively similar.

3. ISOLATED AREAS ON THE LARGE ISLANDS

We have suggested (4) that the remarkably characteristic and constant spot-distribution in the females on the three large islands has been developed in response to the average of the conditions existing in a considerable and diversified habitat. If this is so, we should expect that the isolation, or even partial isolation, of a colony on a small sector of one of the large islands might allow such a local population to achieve an equilibrium different from that which we have shown to be characteristic.

Several such restricted areas in which *M. jurtina* abounds have so far been found and in two of them, one on Tresco and one on St Martin's, spot-distribution data are available for two or more years.

* Individuals with three or more spots are combined to avoid very small or missing values.

(*a*) *The "Farm Area" on Tresco*

In 1954, we found a small and exceptional population occupying an entirely abnormal habitat immediately south of the farm buildings and estate works at New Grimsby, Tresco. Here, a number of huts had been erected during the 1914-18 War. Their concrete foundations remain, and around them has grown up dense vegetation, highly peculiar for the island, of wild plants and garden escapes, which include : *Mesembryanthemum*, *Hyoscyamus*, mullein (*Verbascum*), thistles,

TABLE 10

Spot-distribution in female Maniola jurtina *on the Main and "Farm" Areas of Tresco in 1954 and 1955*

Spots :	0	1	2	3	4	5	Total	Difference
Main Area 1954	26	28	36	5	1	0	96	$\chi^2_{(3)} = 7{\cdot}7$
Farm Area 1954	5	6	18	6	0	0	35	P = 0·05 to 0·1
Main Area 1955	32	31	27	7	4	0	101	$\chi^2_{(3)} = 8{\cdot}10$
Farm Area 1955	23	16	40	6	4	0	89	P = 0·02 to 0·05
Main Area 1954	26	28	36	5	1	0	96	$\chi^2_{(3)} = 3{\cdot}40$
Main Area 1955	32	31	27	7	4	0	101	P = 0·3 to 0·5
Farm Area 1954	5	6	18	6	0	0	35	$\chi^2_{(3)} = 2{\cdot}40$
Farm Area 1955	23	16	40	6	4	0	89	P = 0·3 to 0·5
Main Area Total	*58*	*59*	*63*	*12*	*5*	*0*	197	$\chi^2_{(3)} = 11{\cdot}60$
Farm Area Total	*28*	*22*	*58*	*12*	*4*	*0*	124	P<0·01

long grass, yarrow (*Achillea*), burdock (*Arctium*), yellow-horned poppy (*Glaucium*), bramble, bracken, foxgloves and nettles.

This area occupies only 200×70 yards. It is bounded by the farm buildings to the north and the shore to the west. To the south there is agricultural land unsuited to *M. jurtina* ; in 1955, this was becoming somewhat overgrown. On the eastern side is a road, beyond which is a ditch and hedge bordering the narrow piece of pasture at the edge of the Tresco Lake. This "Farm Area" is connected to our main collecting area by a path bordered by thick bracken and bramble, running to the south along the coast. It is probable that occasional insects migrate along this path, but no considerable flow has been observed, even on days when the species has been abundant.

The abnormal spot-distribution in the Farm Area, which is shown in table 10 was discovered when we first collected from it in 1954. In that year only 35 females were caught. The disparity between this exceptional population and that inhabiting the Main Area of Tresco in 1954 is measured by $\chi^2_{(3)} = 7{\cdot}7$, for which P is just greater

than 0·05. It was obviously of much interest to return to this area in 1955 and it will be seen that the population remained abnormal in this year. The probability of this disparity being due to chance sank below 0·05 with $\chi^2_{(3)} = 8{\cdot}10$ for the 1955 catch. Since the respective spot-distributions of both the Main Area and the Farm Area are homogeneous over the years 1954-55 (table 10), we considered it legitimate to add the data for both years, and this reduced the probability of the disparity in the Farm Area being due to chance to under 0·01 ($\chi^2_{(3)} = 11{\cdot}60$).

Certain changes in the micro-ecology of the Farm Area were noted in 1955, for early in that year a piggery had been installed in the centre of it and the peculiar vegetation had been somewhat reduced and trampled upon. There is at present no indication whether these developments are significant in relation to the spot-distribution of *M. jurtina* or not.

We obtained mark-release-recapture data on the Farm Area population over a period of four days. Analysis of these data by the method of Dowdeswell, Fisher and Ford (1) proved difficult owing to the impossibility of finding a suitable survival ratio. If we had been able to devote a longer period to the study of this population, this difficulty could no doubt have been overcome. Our analyses, unsatisfactory though they proved to be, did nevertheless generally agree with our impression that the daily flying population in the Farm Area was of the order of 100-150 insects.

The results obtained in the Farm Area merit brief consideration in two respects. First, a practical point arises : they stress the extreme necessity in such work of relating the collecting to the ecology of the habitat. Had this sample been combined with the rest of the Tresco insects, an entirely false impression would have been produced ; for the peculiarities of the insects inhabiting this exceptional situation would have been missed, while the total sample would have appeared to diverge from that taken in previous years with which, when restricted to comparable ecological conditions, it is in fact strictly concordant. Secondly, it is necessary to enquire why this population differs from that found throughout the rest of the island. There is a possibility that its numbers are small enough to be affected by genetic " drift ", but the maintenance of the abnormality over two years at least, despite the probability of some leakage from the Main Area, points rather to the existence of some powerful selection pressure peculiar to the Farm Area. If the significant factors causing this pressure are constant, we should expect the population to respond by showing a constant, though divergent, spot-distribution ; if they are being slightly varied, the response may be a gradual swing in the spot-distribution. Such a swing would be dependant on selectional forces, perhaps eventually analysable, rather than on chance alterations in gene-frequencies. We consider it most unlikely to be a coincidence that there is a correspondence between an area which is ecologically

quite exceptional and a population of *M. jurtina* which shows such an unusual structure.

(*b*) *The coastal area on St Martin's opposite White Island*

We have shown on pp. 58 and 59 that there is a large population of *M. jurtina* in the central area of St Martin's whose females have maintained a stable spot-distribution of the "flat-topped" kind, for at least five years. Round the coast of the island, however, there are a few small colonies which are isolated from the central populations by heath moor and extensive areas of bracken. Samples from these isolated populations have been taken and they have been found to diverge in various ways from the standardised female spot-distribution of the central one.

TABLE 11

Spot-distribution in female Maniola jurtina *on the Main Area of St Martin's and on the Coastal Area of St Martin's opposite White Island in the years 1953-55*

Spots :	0	1	2	3	4	5	Total	Difference
1953 Central .	46	34	36	3	1	0	*120*	$\chi^2_{(2)} = 16{\cdot}1$
1953 Coastal .	70	26	13	2	1	0	*112*	$P < 0{\cdot}001$
1954 Central .	76	72	78	8	3	0	*237*	$\chi^2_{(2)} = 11{\cdot}76$
1954 Coastal .	40	14	13	6	1	0	*74*	$P < 0{\cdot}01$
1955 Central .	79	72	68	18	7	0	*244*	$\chi^2_{(2)} = 17{\cdot}0$
1955 Coastal .	30	14	6	0	0	0	*50*	$P < 0{\cdot}001$
1953-5 Coastal .	140	54	32	8	2	0	236	$\chi^2_{(4)} = 6{\cdot}9$ $P = 0{\cdot}1$ to $0{\cdot}2$

Confining our attention to one of these small populations for which we have data for the years 1953-55, we find that within a strip of the St Martin's coast 300 yards long and from 10-80 yards in width, directly opposite White Island, the females of *M. jurtina* have consistently shown a spot-distribution with one large mode at 0. This has not varied appreciably from year to year, and has always been extremely distinct from the population in the centre of St Martin's. Table 11 illustrates this point.

We were at first considerably puzzled by this marked divergence. However, an ecological survey of the surrounding neighbourhood has shown us that isolation of the small coastal strip is indeed effective. Between it and the central region is interposed a waste of bracken, denuded rock and very short heather on podsolized soil. This barrier is about 250 yards wide and stretches to the coast on either side of the restricted area inhabited by *M. jurtina*. Accordingly it seems to us likely that this small coastal population has been able to adjust itself to the special micro-ecological conditions of its limited habitat.

We must also here note, and table 12 demonstrates the fact, that the spot-distribution of the coastal population on St Martin's, opposite White Island, has in no year differed significantly from that of White Island itself. It has already been pointed out (p. 57) that *M. jurtina* must be effectively isolated on White Island. We have no difficulty in rejecting the hypothesis that isolated populations showing similar spot-distributions can arise only through migration. For instance,

TABLE 12

Spot-distribution in female Maniola jurtina *on White Island and on the Coastal Area of St Martin's opposite White Island in the years 1953-55*

Spots :	0	1	2	3	4	5	Total	Difference
1953 White Island	58	30	24	1	0	0	113	$\chi^2_{(2)} = 3{\cdot}38$
1953 St Martin's (Coastal)	70	26	13	2	1	0	112	P = 0·1 to 0·2
1954 White Island	52	26	23	0	0	0	101	$\chi^2_{(2)} = 1{\cdot}24$
1954 St Martin's (Coastal)	40	14	13	6	1	0	74	P = 0·5 to 0·7
1955 White Island	62	34	17	0	0	0	113	$\chi^2_{(2)} = 0{\cdot}441$
1955 St Martin's (Coastal)	30	14	6	0	0	0	50	P = 0·8 to 0·9
1953-5 White Island	172	90	64	1	0	0	327	$\chi^2_{(4)} = 2{\cdot}62$ P = 0·5 to 0·7
1953-5 St Martin's (Coastal)	140	54	32	8	2	0	236	$\chi^2_{(4)} = 6{\cdot}9$ P = 0·1 to 0·2
White Island	172	90	64	1	0	0	327	$\chi^2_{(2)} = 2{\cdot}56$
St Martin's (Coastal)	140	54	32	8	2	0	236	P = 0·2 to 0·3

Tean area 5 and Great Ganilly showed similar spot-distributions in 1951. Yet there are two miles of sea between them and the intervening islands of St Martin's and Arthur show completely dissimilar distributions. Clearly, migration is excluded as the cause of the similarity observed in this case.

It appears that there remain two plausible hypotheses by which we may hope to explain the observed similarity of the White Island population and that confined to the coastal strip of St Martin's opposite White Island. It may be due to an adjustment of both populations to an average of micro-ecological factors which happens to be similar in both habitats. Alternatively, while the operative ecological factors may differ in the two habitats, it may be that the two populations have adjusted themselves to them in ways which chance to have evoked similar spot-distributions. The development of experimental techniques capable of distinguishing between these possibilities is clearly desirable, and it is a project which we hope to undertake.

4. SUMMARY

1. The results of two seasons' work, 1954 and 1955, are summarised in this paper. A distinction is made between Small and Large Islands.

Small islands

2. The removal of cattle from Tean has so altered the ecology of the island that areas which formerly acted as barriers to *M. jurtina* have become colonised by it, and the reverse.

3. The population on Tean area 1 (see map 1) has spread over area 2, yet it has retained its spotting characteristics which have persisted since 1946. Area 3 is now a barrier to the insect. The colony in area 5 has extended over area 4, and its female spot-distribution has completely changed. The reason for this is discussed.

4. Female spotting on Great Ganilly is bimodal. It has shown a slow but significant trend from a greater mode at 0 spots and a smaller mode at 2 in 1951 to the reverse in 1955. No detectable difference in male spotting has taken place over this period.

5. White Island falls within the " small " island group of Scilly. The *M. jurtina* inhabiting it have a female spot-distribution entirely distinct from that of any other small island. This has remained constant since 1953 when it was first examined.

Large islands

6. Further female samples were taken on each of the three " large " islands (St Martin's, St Mary's, Tresco) in 1954 and 1955. The frequency distribution of spotting is the same on these three islands, and has remained so for all the years in which it has been studied.

7. Male spotting has not altered for any island or any year, including St Martin's 1955.

8. Here and there on the large islands small colonies are isolated by barriers from the main population. Two of these have been studied.

9. One of them occupies a small strip of ground on St Martin's opposite White Island. Here female spotting is quite different from that of the main population of St Martin's but identical with that on White Island. It is shown that the latter similarity is not likely to be due to migration.

10. Another such small isolated colony has been found on Tresco. It occupies an ecologically exceptional area, and the female spot-distribution is significantly different from that of the main population of the island. The origin and significance of this difference is discussed.

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LINKAGE IN TRISOMIC INHERITANCE

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1. SUMMARY

APPLICATION of Fisher's general theory of linkage in polysomic inheritance is made to the special case of trisomic inheritance. The enumeration and classification of (1) trisomic genotypes, and (2) monosomic and disomic gametes derived from a trisomic organism is presented. Gametic matrices are then constructed and the estimation procedures for various genetic parameters are outlined. Finally a numerical example is given.

2. INTRODUCTION

The general theory of linkage in polysomic inheritance has been developed primarily by Fisher in two remarkable papers (Fisher, 1947, 1950). Application of the polysomic theory has been made to those organisms in which the number of chromosomes in a set is even, *i.e.* those forms giving rise to tetrasomic, hexasomic, etc. inheritance. The emphasis has been placed on elucidating the inheritance of those species in which "... organisms containing sets of four or six homologous loci are reproduced normally by means of diploid or triploid gametes" (Fisher, 1947). *Lythrum salicaria* is an example of a species normally exhibiting tetrasomic inheritance in which linkage has been investigated (Fisher, 1949).

Earlier studies of linkage in autotetraploids were made by Sömme (1930), De Winton and Haldane (1931), and Sansome (1933). However, the theory of linkage in tetrasomic inheritance was not developed sufficiently at this time to make possible more than approximate estimates of recombination frequencies.

In the present study, application of the general theory developed by Fisher will be made to linkage in trisomic inheritance.

When the number of chromosomes per set is odd, as in trisomic inheritance, meiosis results in at least two classes of gametes, each having different chromosome numbers for a given chromosome set. This usually results in considerable sterility due to genic unbalance in non-homologous chromosomes, and in general, to a lack of stability as a normal mechanism of chromosome transmission. Consequently, trisomic inheritance does not occur as a normal inheritance mechanism.

However, organisms having some or all of their chromosomes in

sets of three have been synthesised and their inheritance studied. These investigations are valuable because they contribute information which is not obtainable in cases of disomic inheritance. Triploid females have been used in linkage studies involving *Drosophila melanogaster* (Bridges and Anderson, 1925, and Redfield, 1930 and 1932), and primary trisomics (designated hereafter as trisomics) have been employed in linkage studies of maize (Rhoades, 1933) and in tomatoes (Lesley, 1937).

The term trisomic is used to designate $2n+1$ chromosomal types in which the chromosomes in one set are in triplicate and in all other sets are in duplicate. Such trisomics are fertile and readily usable in genetic studies. Consistent with the above terminology are the terms monosomic and disomic which we shall use to designate organisms or gametes having one and two chromosomes, respectively, in the given chromosome set.

A trisomic organism produces monosomic and disomic gametes. A complete genetic analysis involves the identification of both gametic series. A single back-cross of the trisomic parent to a completely recessive disomic genotype is all that is necessary to determine the genetic constitution of the monosomic gametes. However, an additional back-cross of the trisomic progeny to the recessive disomic parent is necessary to identify completely the disomic gametes of the original trisomic parent.

Monosomic and disomic gametes can be independently classified into different modes of origin. The frequencies of the modes of gamete origin can be used to estimate the double reduction frequency associated with each locus and the appropriate recombination frequencies. In order that these estimates be unbiased we must assume random disjunction of the chromosomes at meiosis. This assumption will be considered in greater detail in the discussion.

In the following sections we shall consider : (i) the enumeration and classification of trisomic genotypes, (ii) the enumeration and classification of gametes, (iii) the construction of gametic matrices and the estimation of double reduction and recombination frequencies, and (iv) a numerical example.

3. ENUMERATION AND CLASSIFICATION OF TRISOMIC GENOTYPES

(a) Numbers of different possible genotypes

The problem is to compute the number of different genotypes generated by exactly r alleles at the first locus, s alleles at the second locus, etc. To illustrate how this may be done let us consider genotypes involving only two linked loci.

With ρ different alleles at one locus and σ different alleles at the other locus there are $\rho\sigma$ different chromosomal types.

The number of selections of three chromosomes is given by

$$\frac{\rho\sigma(\rho\sigma+1)(\rho\sigma+2)}{3\,!}.$$

By substituting the integers 1, 2 and 3 for ρ and σ, the number of genotypes having ρ or fewer different alleles at one locus and σ or fewer different alleles at the other locus is obtained. These numbers are given in table 1.

TABLE 1

Number of genotypes having ρ or fewer different alleles at one locus and σ or fewer alleles at the other locus

		σ		
		1	2	3
ρ	1	1	4	10
	2	4	20	56
	3	10	56	165

The number of genotypes having *exactly* r different alleles at one locus and *exactly* s at the other can be obtained by operating successively on rows and columns of table 1 with

$$\Delta_\rho^r\ \Delta_\sigma^s\ \frac{\rho\sigma(\rho\sigma+1)(\rho\sigma+2)}{3\,!}\ (\rho = 0,\ \sigma = 0).$$

The resulting numbers are given in table 2.

TABLE 2

Number of genotypes having exactly r different alleles at one locus and s alleles at the other

		s		
		1	2	3
r	1	1	2	1
	2	2	8	6
	3	1	6	6

The above procedure can be extended. However, in the general case the enumeration of isomorphic sets rather than individual genotypes is of primary interest. Therefore we shall defer a generalisation of the enumeration problem until the classification of genotypes into isomorphic sets has been made clear.

(b) *Classification of trisomic genotypes*

Trisomic genotypes involving one, two, or three different alleles at a single locus can be associated with the partitions of the number three as follows :

Type Genetic formula	Partition	Name
$a_1a_1a_1$	(3)	Homogenic
$a_1a_1a_2$ $a_1a_2a_2$	(21)	Digenic (duplex for a_1) Digenic (simplex for a_1)
$a_1a_2a_3$	(1^3)	Trigenic

When two loci are involved, genotypes can be associated with bipartitions of the number three, in which the rows represent the alleles at one locus and the columns represent the alleles at the other locus. Thus, for the genotype $(a_1b_1)(a_2b_2)(a_3b_3)$ the associated bipartition is

	b_1	b_2	b_3	
a_1	1	.	.	
a_2	.	1	.	(1^3)
a_3	.	.	1	
		(1^3)		

Genotypes may be classified into isomorphic sets such that isomorphic genotypes are those which can be derived one from the other by permutation of genes at the same locus.

Experimentally, classification of genotypes into isomorphic sets is important. Most genotypes yield gametic matrices which are deficient in certain respects. Genotypes in the same isomorphic set have equivalent gametic matrices and, therefore, use of different isomorphic genotypes will not alter the elements of deficiency. However, genotypes in different isomorphic sets have different gametic matrices and use of non-isomorphic genotypes may reduce the elements of deficiency.

Isomorphic sets can be related by permutation of loci. Thus, conjugate pairs of sets are those in which one set can be derived from the other by permutation of loci, and a self-conjugate set is one which is transformed into itself by this operation.

To illustrate the classification of genotypes, let us consider those genotypes which are digenic or trigenic at each of two loci. A detailed list is given in table 3.

The eight genotypes digenic at both loci can be classified into two isomorphic, self-conjugate sets of four genotypes each. The six genotypes digenic at "a" locus and trigenic at "b" locus constitute

TABLE 3

Classification of genotypes which are digenic or trigenic at each of two loci

Type genotype	Bipartition	No. of genotypes	No. of isomorphic sets
(1) Genotypes digenic for both loci—			
$(a_1b_1)_2(a_2b_2)$	2 . . 1	4	1 (self-conjugate)
$(a_1b_2)(a_2b_1)(a_2b_2)$ . . .	. 1 1 1	4	1 (self-conjugate) — 2
(2) Genotypes digenic at one locus and trigenic at the other—			
$(a_1b_1)(a_2b_2)(a_2b_3)$ . . .	1 . . 1 . 1	6	1 — (conjugate sets)
$(a_1b_1)(a_2b_2)(a_3b_3)$ . . .	1 . . . 1 1	6	1 — (conjugate sets)
(3) Genotypes trigenic at both loci—			
$(a_1b_1)(a_2b_2)(a_3b_3)$ . . .	1 . . . 1 . . . 1	6	1 (self-conjugate)

TABLE 4

Number of isomorphic sets of genotypes having exactly r *different alleles at one locus and* s *at the other*

r \ s	1	2	3	
1	1	1	1	
2	1	2	1	
3	1	1	1	
				10

one isomorphic set. This set is conjugate to the set of six genotypes trigenic at the "a" locus and digenic at the "b" locus. The genotypes trigenic at both loci constitute a self-conjugate set. Thus the genotypes involving two loci and having exactly r alleles at one locus and s alleles at the second, as given in table 2, can be classified into isomorphic sets, the numbers of which are given in table 4.

Adapting the enumeration procedure given by Fisher (1950) to trisomics we find that the numbers of isomorphic sets for various classes of genotypes can be enumerated for any number, l say, of loci by the following formulæ :

Genotypes digenic for all loci,

$$A = \tfrac{1}{2}(1+3^{l-1}) ;$$

Genotypes digenic and/or trigenic,

$$B = \tfrac{1}{6}(2+3\cdot 2^{l}+4^{l}) ;$$

All genotypes,

$$C = \tfrac{1}{6}(2\cdot 2^{l}+3\cdot 3^{l}+5^{l}).$$

Using these formulæ the following numbers of isomorphic sets are found for l up to six :

l	A	B	C
1	1	2	3
2	2	5	10
3	5	15	37
4	14	51	150
5	41	187	653
6	122	715	2990

4. ENUMERATION AND CLASSIFICATION OF GAMETES

Trisomic genotypes give rise to monosomic and disomic gametes. We shall consider each class separately. Within each class the gametes can be grouped according to their mode of origin. Gametes are said to belong to the same mode of gamete origin if they can be derived one from another by permutation of chromosomes. Since a set of three chromosomes is involved, a symmetric permutation group of degree three (denoted P_3) can be used to classify the gametes into their respective modes.

(a) *Monosomic gametes*

(i) *Single locus.* The number of different gametic types is obviously equal to the number of different alleles in the parent genotype. The expected gametic ratio is the same as the ratio of alleles in the parent genotype : *i.e.* the genotype $a_1a_1a_2$ gives rise to the expected gametic ratio of $2(a_1) : 1(a_2)$. There is only one mode of gamete formation.

(ii) *Two linked loci.* With exactly r different alleles at one locus and s different alleles at the other, there are rs different chromosomal combinations which in turn are equivalent to the monosomic gametic genotypes. These gametes can be classified into different modes by use of the permutation group P_3.

For example, if the genotype is trigenic for each locus, *i.e.* $(a_1b_1)(a_2b_2)(a_3b_3)$, nine different monosomic gametic types may be

produced. These are classified into two modes of gamete formation as follows :

Permutation	Mode 1	Mode 2
I	a_1b_1	a_1b_2
(12)	a_2b_2	a_2b_1
(13)	a_3b_3	a_3b_2
(23)	a_1b_1	a_1b_3
(123)	a_3b_3	a_3b_1
(132)	a_2b_2	a_2b_3

It is clear that mode 1 represents non-recombinant and mode 2 represents recombinant chromosomal (gametic) types.

(iii) *Three linked loci.* Assume that the loci are in the linear order *a-b-c* and that the chromosomal segments between loci " a " and " b " and between " b " and " c " are designated regions 1 and 2, respectively. A triply trigenic genotype may produce 27 different possible types of gametes, which can be classified into five gametic

TABLE 5

Classification of monosomic gametes from the genotype $(a_1b_1c_1)$ $(a_2b_2c_2)$ $(a_3b_3c_3)$ *into five gametic modes*

Mode	Gamete type	No. of gametes	Genetic interpretation
1	$a_1b_1c_1$	3	Non-recombinant
2	$a_1b_2c_2$	6	Recombination in region 1 only
3	$a_1b_1c_2$	6	Recombination in region 2 only
4	$a_1b_2c_1$	6	Simultaneous recombination in regions 1 and 2 involving two chromosomes (recurrent)
5	$a_1b_2c_3$	6	Simultaneous recombination in regions 1 and 2 involving three chromosomes (progressive)

modes. This classification together with the genetic interpretation associated with each mode is given in table 5.

(iv) *Four linked loci.* With four linked loci in the linear order *a-b-c-d* there are 81 different possible gametic types from the genotype $(a_1b_1c_1d_1)(a_2b_2c_2d_2)(a_3b_3c_3d_3)$, which can be classified into 14 modes. This classification together with the genetic interpretation associated with each mode is given in table 6.

In general, the number of modes of gamete formation for any given number, l say, of loci is given by the formula

$$D = \tfrac{1}{2}(1+3^{l-1}).$$

This formula is the same as that enumerating the number of isomorphic sets of genotypes which are digenic for all loci.

(b) *Disomic gametes*

(i) *Single locus.* A trisomic genotype having three different genes at a given locus is capable of producing six different disomic gametic types, namely, a_1a_1, a_2a_2, a_3a_3, a_1a_2, a_1a_3, and a_2a_3. The "homozygous" gametes, resulting from double reduction, constitute one mode, and the "heterozygous" gametes constitute the other.

TABLE 6

Classification of monosomic gametes from the genotype $(a_1b_1c_1d_1)$ $(a_2b_2c_2d_2)$ $(a_3b_3c_3d_3)$ *into 14 gametic modes*

Mode	Gamete type	No. of gametes	Genetic interpretation
1	$a_1b_1c_1d_1$	3	Non-recombinant
2	$a_1b_2c_2d_2$	6	Recombination in region 1 only
3	$a_1b_1c_2d_2$	6	Recombination in region 2 only
4	$a_1b_1c_1d_2$	6	Recombination in region 3 only
5	$a_1b_2c_1d_1$	6	Recombination in regions 1 and 2 (2 chromosomes involved)
6	$a_1b_2c_3d_3$	6	Recombination in regions 1 and 2 (3 chromosomes involved)
7	$a_1b_2c_2d_1$	6	Recombination in regions 1 and 3 (2 chromosomes involved)
8	$a_1b_2c_2d_3$	6	Recombination in regions 1 and 3 (3 chromosomes involved)
9	$a_1b_1c_2d_1$	6	Recombination in regions 2 and 3 (2 chromosomes involved)
10	$a_1b_1c_2d_3$	6	Recombination in regions 2 and 3 (3 chromosomes involved)
11	$a_1b_2c_1d_2$	6	Recombination in regions 1, 2 and 3 (2 chromosomes involved)
12	$a_1b_2c_1d_3$	6	Recombination in regions 1, 2 and 3 (3 chromosomes involved)
13	$a_1b_2c_3d_1$	6	Recombination in regions 1, 2 and 3 (3 chromosomes involved)
14	$a_1b_2c_3d_2$	6	Recombination in regions 1, 2 and 3 (3 chromosomes involved)

(ii) *Two linked loci.* If at two linked loci there are r different genes at one locus and s different genes at the other, there are rs different chromosomal types. These may give rise to a possible number of $\frac{rs(rs+1)}{2}$ different disomic gametes.

For example, the genotype $(a_1b_1)(a_2b_2)(a_3b_3)$ yields nine different chromosomal types which, taken in pairs, form 45 different possible disomic gametes. These can be classified into 10 gametic modes by the permutation group, P_3. Table 7 lists the modes together with the gametic information which can be obtained from them. These modes have been numbered so as to correspond to the tetrasomic modes (Fisher, 1947), except that, since tetrasomic mode six does not occur in trisomics, the trisomic mode six corresponds to tetrasomic mode 7, etc.

In general, the number of modes of gamete formation for any given number, l say, of loci is given by the formula

$$E = \tfrac{1}{12}(9^l + 4 \cdot 3^l + 3),$$

which yields the following values for l up to 6 :

l	1	2	3	4	5	6
E	2	10	70	574	5002	44530

TABLE 7

Classification of disomic gametes from the genotype $(a_1b_1)(a_2b_2)(a_3b_3)$ *into 10 gametic modes*

Mode	Gamete type	No. of gametes	Recombinant chromosomes	Double reduction at	
				" a " locus	" b " locus
1	$(a_1b_1)(a_2b_3)$	3	0	−	−
2	$(a_1b_1)(a_1b_1)$	3	0	+	+
3	$(a_1b_1)(a_2b_3)$	6	$\frac{1}{2}$	−	−
4	$(a_1b_1)(a_1b_2)$	6	$\frac{1}{2}$	+	−
5	$(a_1b_1)(a_2b_1)$	6	$\frac{1}{2}$	−	+
6	$(a_2b_1)(a_3b_1)$	3	1	−	+
7	$(a_1b_2)(a_1b_3)$	3	1	+	−
8	$(a_1b_2)(a_2b_3)$	6	1	−	−
9	$(a_1b_2)(a_1b_2)$	6	1	+	+
10	$(a_1b_2)(a_2b_1)$	3	1	−	−

5. GAMETIC MATRICES AND THE ESTIMATION OF GENETIC PARAMETERS

We have seen that a trisomic genotype gives rise to monosomic and disomic gametes. These two classes of gametes can be treated independently. Within each class the gametes are assigned to modes of gamete formation. We are concerned with the frequencies of these modes of gamete formation since it is clear that recombination and double reduction parameters are functions of the gametic mode frequencies. For example, consider genotypes at two loci. If all gametes are identifiable it is obvious that

(a) for monosomic gametes

$$y_{ab} = f_2, \text{ and}$$

(b) for disomic gametes

$$y_{ab} = \tfrac{1}{2}(f_3 + f_4 + f_5) + f_6 + f_7 + f_8 + f_9 + f_{10}$$
$$\alpha_a = f_2 + f_4 + f_7 + f_9$$
$$\alpha_b = f_2 + f_5 + f_6 + f_9,$$

where y_{ab} is the recombination frequency for the chromosomal segment between the " a " and " b " loci, f_i is the frequency of the i^{th} mode, and α_j is the double reduction frequency for the j^{th} locus.

The estimation of genetic parameters from either class of gametes is briefly outlined as follows :

Denote the frequencies of gametic modes by the row vector $F = (f_1, f_2, \ldots\ldots)$, and the observed gametic numbers by the vector $A = (a_1, a_2, \ldots\ldots)$, where the total number of gametes identified is $N = \sum_i a_i$. A gametic matrix $\{g_{ij}\}$ is defined in which the rows correspond to modes and the columns correspond to gametic genotypes in such a way that the elements in the i^{th} row are the expected gametic frequencies for the i^{th} mode. The expected numbers for the different gametes are given by the matrix equation $F \cdot G = M = \{m_k\}$, where $G = N\{g_{ij}\}$.

If the rank of G is equal to the number of different modes, maximum likelihood estimates of the frequencies F are possible. This is usually the case for those monosomic gametic matrices of interest. However, it is usually not the case for the disomic gametic matrices.

To examine more closely the situation with disomic gametic matrices, let us consider those matrices arising from genotypes involving genes at two loci.

If the rank of G is 10, it is possible to estimate all f_i from the experimental data. However, this is true only for the genotype $(a_1b_1)(a_2b_2)(a_3b_3)$.

If the rank of G is less than 10 {r say, where $r<10$} then a deficiency matrix, D, of rank $(10-r)$ is associated with G such that

$$D_{(10-r)\times 10} \cdot G_{10\times n} = O_{(10-r)\times n},$$

where n is the number of different gametes. To determine estimable functions of f_i we define a matrix T of rank r such that

$$D_{(10-r)\times 10} \cdot T_{10\times r} = O_{(10-r)\times r}.$$

The estimable functions are then defined as $U_{1\times r} = F_{1\times 10} \cdot T_{10\times r}$.

A new gametic matrix $G^* = N\{g^*_{ij}\}$ is constructed such that $U_{1\times r} \cdot G^*_{r\times n} = M_{1\times n}$, where M is the row vector of expected gametic numbers. Then, given the observed gametic frequencies $A = (a_1, a_2, \ldots\ldots)$, it is possible to calculate the maximum likelihood estimates $\hat{U}_{1\times r}$ (see Fisher, 1947). In all cases which will be discussed, it is possible to estimate recombination and double reduction parameters by use of $\hat{U}_{1\times r}$.

In those genotypes trigenic for all loci, each gametic type is produced by one, and only one, mode of gamete formation. In this case the estimation of mode frequencies is extremely simple in that the i^{th} mode frequency is estimated as the summation of frequencies of all observable gametic types due to the i^{th} mode of origin.

However, with all other genotypes certain gametic types may result from two or more different gametic modes, and a more complicated procedure is necessary to obtain maximum likelihood estimates. This procedure is outlined and illustrated in detail by Fisher (1947) and,

therefore, will not be given here. Suffice it to say that the procedure is an iterative one in which it is necessary to invert a square information matrix of dimensions $n \times n$, where n is one less than the rank of the gametic matrix involved. Because of computational difficulties, the size of the gametic matrix which can be inverted is limited. For this reason we shall give examples of monosomic gametic matrices for genotypes involving up to four loci, and examples of disomic gametic matrices for genotypes involving only two loci.

Methods by means of which a considerable number of loci may be included in a given study are suggested in the discussion.

(*a*) *Monosomic gametic matrices*

(i) *Single locus.* Trisomic genotypes involving alleles at a single locus yield monosomic gametes all of which can be classified into a single mode of gamete formation.

Data arising from monosomic gametes may be utilised for making " goodness of fit " tests. In this case the observed numbers of different disomic backcross progeny are compared with expected numbers based upon the frequencies of the alleles in the parental genotype. Discrepancies are probably due to viability differences associated directly with the alleles or to closely linked genes having lowered viability effects.

(ii) *Two linked loci.* The genotype $(a_1b_1)(a_2b_2)(a_3b_3)$ gives rise to the monosomic gametic matrix given in table 8.

TABLE 8

Monosomic gametic matrix for the genotype $(a_1b_1)(a_2b_2)(a_3b_3)$

Mode	Gametes								
	a_1b_1	a_2b_2	a_3b_3	a_1b_2	a_1b_3	a_2b_3	a_2b_1	a_3b_1	a_3b_2
1	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	...	...	...	...	...	...
2	...	...	...	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$

Corresponding gametic matrices may be formed for other genotypes. Of particular interest are the gametic matrices for the genotypes $(a_1b_1)_2(a_2b_2)$ and $(a_1b_1)(a_1b_2)(a_2b_1)$. These are given in tables 9 and 10. Both of these matrices are of rank two and, therefore, gametic mode frequencies can be estimated.

(iii) *Three linked loci.* The monosomic gametic matrix for the genotype $(a_1b_1c_1)(a_2b_2c_2)(a_3b_3c_3)$ consists of five rows corresponding to the five modes and 27 columns corresponding to the 27 different possible gametes.

The entries in the matrix may be described as follows :

Mode 1 : frequency of $\frac{1}{3}$ for gametes of type $a_1b_1c_1$ and zero elsewhere ;

Mode 2 : frequency of $\frac{1}{6}$ for gametes of type $a_1b_2c_2$ and zero elsewhere ;

Mode 3 : frequency of $\frac{1}{6}$ for gametes of type $a_1b_1c_2$ and zero elsewhere ;

Mode 4 : frequency of $\frac{1}{6}$ for gametes of type $a_1b_2c_1$ and zero elsewhere ; and,

Mode 5 : frequency of $\frac{1}{6}$ for gametes of type $a_1b_2c_3$ and zero elsewhere.

TABLE 9

Monosomic gametic matrix for the genotype $(a_1b_1)_2(a_2b_2)$

Mode	Gametes			
	a_1b_1	a_2b_2	a_1b_2	a_2b_1
1	$\frac{2}{3}$	$\frac{1}{3}$	...	...
2	$\frac{1}{3}$	...	$\frac{1}{3}$	$\frac{1}{3}$

TABLE 10

Monosomic gametic matrix for the genotype $(a_1b_1)(a_1b_2)(a_2b_1)$

Mode	Gametes			
	a_1b_1	a_1b_2	a_2b_1	a_2b_2
1	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	...
2	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$

This matrix is of rank five and therefore gametic mode frequencies can be estimated.

Gametic matrices for other genotypes may be easily derived from the one given above. Of particular interest is that for the genotype $(a_2b_1c_1)(a_1b_2c_1)(a_1b_1c_2)$. This matrix, which is set out in table 11, is needed for the computational example given in Section V. It is of rank five and, therefore, the mode frequencies can be estimated.

(*b*) *Disomic gametic matrices*

(i) *Single locus.* The gametic matrix for the genotype $a_1a_2a_3$ is given in table 12. The homozygous gametes a_1a_1 arise from a common

mode of gamete formation and are due to the phenomenon of double reduction.

This matrix is of rank two, and therefore if the gametic types can

TABLE 11

Monosomic gametic matrix for the genotype $(a_1b_1c_1)(a_1b_2c_1)(a_1b_1c_2)$

Mode	Gametes							
	$a_1b_1c_1$	$a_1b_1c_2$	$a_1b_2c_1$	$a_1b_2c_2$	$a_2b_1c_1$	$a_2b_2c_1$	$a_2b_2c_2$	$a_2b_1c_2$
1	...	$\frac{1}{3}$	$\frac{1}{3}$	...	$\frac{1}{3}$	...	...	...
2	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	...	...	$\frac{1}{6}$	...	$\frac{1}{6}$
3	$\frac{1}{3}$	...	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	...	...	$\frac{1}{6}$
4	$\frac{1}{3}$	$\frac{1}{6}$	...	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	...	...
5	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	...	$\frac{1}{6}$	...	$\frac{1}{6}$	...

TABLE 12

Disomic gametic matrix for the genotype $a_1a_2a_3$

Mode	Gametes					
	a_1a_1	a_2a_2	a_3a_3	a_1a_2	a_1a_3	a_2a_3
1	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	...	...	...
2	...	...	...	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$

TABLE 13

Disomic gametic matrix for the genotype $a_1a_1a_2$

Mode	Gametes		
	a_1a_1	a_2a_2	a_1a_2
1	$\frac{2}{3}$	$\frac{1}{3}$	...
2	...	...	1

be identified, there is no problem in estimating the mode frequencies and hence the frequency of double reduction.

However, the estimation procedure usually involves digenic types in which one allele is dominant to the other. If a_1 is dominant to a_2, the appropriate cross for estimating double reduction frequency is $a_1a_1a_2 \times a_2a_2$. The disomic gametic matrix for $a_1a_1a_2$ is given in table 13, and the corresponding phenotypic matrix associated with

the backcross progeny is given in table 14. If we let α represent the frequency of double reduction, and if n is the number of observed recessive phenotypes occurring in a total number of N trisomic progeny, then α is estimated by $\hat{\alpha} = \frac{3n}{N}$.

(ii) *Genotypes involving two loci.* The genotype having three different alleles at each of two linked loci, *i.e.* $(a_1b_1)(a_2b_2)(a_3b_3)$ has a disomic gametic matrix of 10 rows (each corresponding to a mode of gamete

TABLE 14

Phenotypic matrix for trisomic progeny from the cross $a_1a_1a_2 \times a_2a_2$

Mode	Phenotypes	
	recessive $a_2a_2a_2$	dominant a_1____
1	$\frac{1}{3}$	$\frac{2}{3}$
2	...	1

formation) and 45 columns (one for each gametic type). The entries, given for each row, may be described as follows :

Mode 1 : frequency of $\frac{1}{3}$ for gametes of type $(a_1b_1)(a_2b_2)$ and zero elsewhere.

Mode 2 : frequency of $\frac{1}{3}$ for gametes of type $(a_1b_1)(a_1b_1)$ and zero elsewhere.

Mode 3 : frequency of $\frac{1}{6}$ for gametes of type $(a_1b_1)(a_2b_3)$ and zero elsewhere.

Mode 4 : frequency of $\frac{1}{6}$ for gametes of type $(a_1b_1)(a_1b_2)$ and zero elsewhere.

Mode 5 : frequency of $\frac{1}{6}$ for gametes of type $(a_1b_1)(a_2b_1)$ and zero elsewhere.

Mode 6 : frequency of $\frac{1}{3}$ for gametes of type $(a_2b_1)(a_3b_1)$ and zero elsewhere.

Mode 7 : frequency of $\frac{1}{3}$ for gametes of type $(a_1b_2)(a_1b_3)$ and zero elsewhere.

Mode 8 : frequency of $\frac{1}{6}$ for gametes of type $(a_1b_2)(a_2b_3)$ and zero elsewhere.

Mode 9 : frequency of $\frac{1}{6}$ for gametes of type $(a_1b_2)(a_1b_2)$ and zero elsewhere.

Mode 10 : frequency of $\frac{1}{3}$ for gametes of type $(a_1b_2)(a_2b_1)$ and zero elsewhere.

Since the rank of this matrix is 10, gametic mode frequencies can be estimated.

TABLE 15

Disomic gametic matrix for the genotype $(a_1b_1)(a_2b_2)(a_3b_2)$

Mode	$\frac{a_1b_1}{a_1b_1}$	$\frac{a_1b_2}{a_1b_2}$	$\frac{a_2b_1}{a_2b_1}$	$\frac{a_2b_2}{a_2b_2}$	$\frac{a_3b_1}{a_3b_1}$	$\frac{a_3b_2}{a_3b_2}$	$\frac{a_1b_1}{a_1b_2}$	$\frac{a_2b_1}{a_2b_2}$	$\frac{a_3b_1}{a_3b_2}$	$\frac{a_1b_1}{a_2b_1}$	$\frac{a_1b_2}{a_2b_2}$	$\frac{a_1b_1}{a_3b_1}$	$\frac{a_1b_2}{a_3b_2}$	$\frac{a_2b_1}{a_3b_1}$	$\frac{a_2b_2}{a_3b_2}$	$\frac{a_1b_1}{a_2b_2}$	$\frac{a_1b_2}{a_2b_1}$	$\frac{a_1b_1}{a_3b_2}$	$\frac{a_1b_2}{a_3b_1}$	$\frac{a_2b_1}{a_3b_2}$	$\frac{a_2b_2}{a_3b_1}$
1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.
2	$\frac{1}{3}$	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	$\frac{1}{6}$	.	$\frac{1}{6}$	.	.	$\frac{1}{6}$	.	$\frac{1}{6}$	.	$\frac{1}{6}$	$\frac{1}{6}$
4	.	.	.	$\frac{1}{6}$	.	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	.	$\frac{1}{3}$	.	.	.	.	.	.
6	.	.	.	.	.	.	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	.	.	.	.
7	.	$\frac{1}{3}$	.	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	.	.	.	.	.	.	.	.	.
8	.	.	.	.	.	.	.	.	.	.	$\frac{1}{6}$	.	$\frac{1}{6}$	.	.	.	$\frac{1}{6}$	.	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$
9	.	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.

$D \equiv [1 \cdot -2 \cdot \ldots \cdot 2 \cdot -1]$

Genotypes digenic at one locus and trigenic at the other yield gametic matrices having the dimensions of 10 rows by 21 columns. Such a matrix for the genotype $(a_1b_1)(a_2b_2)(a_3b_2)$ is given in table 15. Since the rank of this matrix is nine there is a deficiency row vector, D, associated with it. A set of estimable variables $U = (u_1 \ldots\ldots u_9)$ may be defined. The functional relationships of $U_{1\times9}$ and $F_{1\times10}$ are set out in table 16. The gametic matrix $G^*_{9\times21}$ of rank nine, associated with the $U_{1\times9}$ variables is given in table 17. Maximum likelihood estimates, $\hat{U}_{1\times9}$, can be obtained using the observed gametic numbers $A = (a_1, a_2 \ldots\ldots a_{21})$.

TABLE 16

A set of estimable variables $U_{1\times9}$ as functions of the gametic mode frequencies $F_{1\times10}$ for the genotype $(a_1b_1)(a_2b_2)(a_3b_2)$

	f_1	f_2	f_3	f_4	f_5	f_6	f_7	f_8	f_9	f_{10}
u_1	1	.	$\frac{1}{2}$	.	.	.	.	.	.	.
u_2	.	1	.	.	.	.	.	.	.	.
u_3	.	.	$\frac{1}{2}$	.	.	.	.	$\frac{1}{2}$	.	.
u_4	.	.	.	1	.	.	.	.	.	.
u_5	.	.	.	.	1	.	.	.	.	.
u_6	.	.	.	.	.	1	.	.	.	.
u_7	.	.	.	.	.	.	1	.	.	.
u_8	.	.	.	.	.	.	.	$\frac{1}{2}$	.	1
u_9	.	.	.	.	.	.	.	.	1	.

Double reduction and recombination frequencies can be estimated from $\hat{U}$ as follows :

$$\hat{a}_a = \hat{u}_2+\hat{u}_4+\hat{u}_7+\hat{u}_9$$
$$\hat{a}_b = \hat{u}_2+\hat{u}_5+\hat{u}_6+\hat{u}_9$$
$$\hat{y}_{ab} = \tfrac{1}{2}(2\hat{u}_3+\hat{u}_4+\hat{u}_5)+\hat{u}_6+\hat{u}_7+\hat{u}_8+\hat{u}_9.$$

Genotypes digenic at each of two loci can be classified into two isomorphic sets. It is of interest to examine disomic gametic matrices for at least two non-isomorphic genotypes. We choose the genotypes $(a_1b_1)_2(a_2b_2)$ and $(a_1b_1)(a_1b_2)(a_2b_1)$.

The genotype $(a_1b_1)_2(a_2b_2)$ has a disomic gametic matrix of rank eight and, therefore, an associated deficiency matrix of rank two. These are given in table 18. The estimable variables $U_{1\times8}$ are given in table 19 as functions of $F_{1\times10}$. The gametic matrix $G^*_{6\times10}$ of rank eight associated with the variables $U_{1\times8}$ is given in table 20. Double reduction and recombination estimates are obtained from $\hat{U}$ as follows :

$$\hat{a}_a = \hat{u}_2+\hat{u}_4+\hat{u}_7$$
$$\hat{a}_b = \hat{u}_2+\hat{u}_5+\hat{u}_6+\hat{u}_7$$
$$\hat{y}_{ab} = \tfrac{1}{2}(\hat{u}_3+\hat{u}_4+\hat{u}_5)+\hat{u}_6+\hat{u}_7+\hat{u}_8.$$

The disomic gametic matrix of rank eight and its associated deficiency matrix of rank two for the genotype $(a_1b_1)(a_1b_2)(a_2b_1)$ are

TABLE 17

Disomic gametic matrix associated with the $U_{1\times 9}$ variables for the genotype $(a_1b_1)(a_2b_2)(a_3b_2)$

	$\frac{a_1b_1}{a_1b_1}$	$\frac{a_1b_2}{a_1b_2}$	$\frac{a_2b_1}{a_2b_1}$	$\frac{a_2b_2}{a_2b_2}$	$\frac{a_3b_1}{a_3b_1}$	$\frac{a_3b_2}{a_3b_2}$	$\frac{a_1b_1}{a_1b_2}$	$\frac{a_2b_1}{a_2b_2}$	$\frac{a_3b_1}{a_3b_2}$	$\frac{a_1b_1}{a_2b_1}$	$\frac{a_1b_2}{a_2b_2}$	$\frac{a_1b_1}{a_3b_1}$	$\frac{a_1b_2}{a_3b_2}$	$\frac{a_2b_1}{a_3b_1}$	$\frac{a_2b_2}{a_3b_2}$	$\frac{a_1b_1}{a_2b_2}$	$\frac{a_1b_2}{a_2b_1}$	$\frac{a_1b_1}{a_3b_2}$	$\frac{a_1b_2}{a_3b_1}$	$\frac{a_2b_1}{a_3b_2}$	$\frac{a_2b_2}{a_3b_1}$
u_1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.
u_2	$\frac{1}{3}$	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
u_3	.	.	.	.	.	.	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	$-\frac{1}{3}$	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$
u_4	.	.	.	$\frac{1}{6}$	.	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	.	.	.	.	.	.	.	.	.
u_5	.	.	.	.	.	.	.	.	.	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	.	$\frac{1}{3}$	.	.	.	.	.	.
u_6	.	.	.	.	.	.	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	.	.	.	.
u_7	.	$\frac{1}{3}$	.	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	.	.	.	.	.	.	.	.	.
u_8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.
u_9	.	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TABLE 18

Disomic gametic matrix for the genotype $(a_1b_1)_2(a_2b_2)$

Mode	Gametes									
	$\frac{a_1b_1}{a_1b_1}$	$\frac{a_2b_1}{a_2b_1}$	$\frac{a_2b_2}{a_2b_2}$	$\frac{a_1b_2}{a_1b_2}$	$\frac{a_1b_1}{a_1b_2}$	$\frac{a_2b_1}{a_1b_1}$	$\frac{a_2b_2}{a_2b_1}$	$\frac{a_1b_2}{a_2b_2}$	$\frac{a_1b_1}{a_2b_2}$	$\frac{a_1b_2}{a_2b_1}$
1	$\frac{1}{3}$	.	.	.	.	.	.	.	$\frac{2}{3}$	.
2	$\frac{2}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.	.
3	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	$\frac{1}{3}$	.
4	$\frac{1}{3}$	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.
5	$\frac{1}{3}$	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.
6	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.	.
7	.	$\frac{2}{3}$	.	.	$\frac{2}{3}$	.	.	.	.	.
8	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	$\frac{1}{3}$
9	$\frac{1}{3}$	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.
10	$\frac{1}{3}$	.	.	.	.	.	.	.	.	$\frac{2}{3}$

$$D = \begin{bmatrix} 1 & . & -2 & . & . & 1 & 1 & . & -1 & . \\ 1 & . & -2 & . & . & . & . & 2 & . & -1 \end{bmatrix}$$

TABLE 19

A set of estimable variables, $U_{1\times9}$ *as functions of gametic mode frequencies,* $F_{1\times10}$ *for the genotype* $(a_1b_1)_2(a_2b_2)$

	f_1	f_2	f_3	f_4	f_5	f_6	f_7	f_8	f_9	f_{10}
u_1	1	.	.	.	.	.	-1	$-\frac{1}{2}$	.	.
u_2	.	1	.	.	.	.	.	.	.	.
u_3	.	.	1	.	.	.	2	1	.	.
u_4	.	.	.	1	.	.	.	.	.	.
u_5	.	.	.	.	1	.	.	.	.	.
u_6	.	.	.	.	.	1	-1	.	.	.
u_7	.	.	.	.	.	.	1	.	1	.
u_8	.	.	.	.	.	.	.	$\frac{1}{2}$	.	1

TABLE 20

Disomic gametic matrix associated with the $U_{1\times9}$ *variables for the genotype* $(a_1b_1)_2(a_2b_2)$

	Gametes									
	$\frac{a_1b_1}{a_1b_1}$	$\frac{a_2b_1}{a_2b_1}$	$\frac{a_2b_2}{a_2b_2}$	$\frac{a_1b_2}{a_1b_2}$	$\frac{a_1b_1}{a_1b_2}$	$\frac{a_2b_1}{a_1b_1}$	$\frac{a_2b_2}{a_2b_1}$	$\frac{a_1b_2}{a_2b_2}$	$\frac{a_1b_1}{a_2b_2}$	$\frac{a_1b_2}{a_2b_1}$
u_1	$\frac{1}{3}$	.	.	.	.	.	.	.	$\frac{2}{3}$	.
u_2	$\frac{2}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.	.
u_3	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	$\frac{1}{3}$	.
u_4	$\frac{1}{3}$	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.
u_5	$\frac{1}{3}$	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.
u_6	.	.	.	$\frac{1}{3}$	.	$\frac{2}{3}$	.	.	.	.
u_7	$\frac{1}{3}$	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.
u_8	$\frac{1}{3}$	.	.	.	.	.	.	.	.	$\frac{2}{3}$

given in table 21. The variables $U_{1\times 8}$ as functions of $F_{1\times 10}$ are given in table 22, and the gametic matrix, $G^*_{8\times 10}$ associated with $U_{1\times 8}$ is given in table 23.

TABLE 21

Disomic gametic matrix for the genotype $(a_1b_1)(a_1b_2)(a_2b_1)$

Mode	Gametes									
	$\frac{a_1b_1}{a_1b_1}$	$\frac{a_2b_1}{a_2b_1}$	$\frac{a_2b_2}{a_2b_2}$	$\frac{a_1b_2}{a_1b_2}$	$\frac{a_1b_1}{a_1b_2}$	$\frac{a_2b_1}{a_1b_1}$	$\frac{a_2b_2}{a_2b_1}$	$\frac{a_1b_2}{a_2b_2}$	$\frac{a_1b_1}{a_2b_2}$	$\frac{a_1b_2}{a_2b_1}$
1	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	$\frac{1}{3}$
2	$\frac{1}{3}$	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.
3	$\frac{1}{6}$	.	.	.	$\frac{1}{6}$	$\frac{1}{6}$	.	.	$\frac{1}{6}$	$\frac{1}{6}$
4	$\frac{1}{6}$	$\frac{1}{6}$	.	.	$\frac{1}{2}$	.	$\frac{1}{6}$	.	.	.
5	$\frac{1}{6}$	.	.	$\frac{1}{6}$	.	$\frac{1}{2}$	.	$\frac{1}{6}$	.	.
6	$\frac{1}{3}$	.	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.
7	$\frac{1}{3}$	.	.	.	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.
8	$\frac{1}{6}$	.	.	.	$\frac{1}{6}$	$\frac{1}{6}$	.	.	$\frac{1}{3}$	$\frac{1}{6}$
9	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	.	.	.	.	.	.
10	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	$\frac{1}{3}$	.

$$D \equiv \begin{bmatrix} -2 & -1 & 2 & 2 & 2 & -1 & -1 & . & . & -1 \\ -1 & . & 2 & . & . & . & . & -2 & . & 1 \end{bmatrix}$$

TABLE 22

A set of estimable variables, $U_{1\times 8}$ *as functions of gametic mode frequencies,* $F_{1\times 10}$, *for the genotype* $(a_1b_1)(a_1b_2)(a_2b_1)$

	f_1	f_2	f_3	f_4	f_5	f_6	f	f_8	f_9	f_{10}
u_1	1	.	.	.	1	−1	.	.	.	1
u_2	.	1	.	.	.	.	−1	.	.	.
u_3	.	.	1	.	.	.	2	1	.	.
u_4	.	.	.	1	−1	.	.	.	.	.
u_5	.	.	.	.	1	1	1	.	.	.
u_6	.	.	.	.	.	1	−2	$\frac{1}{2}$	.	1
u_7	.	.	.	.	.	.	1	$-\frac{1}{2}$	.	−1
u_8	.	.	.	.	.	.	.	.	1	.

TABLE 23

Disomic gametic matrix associated with the $U_{1\times 8}$ *variables for the genotype* $(a_1b_1)(a_1b_2)(a_2b_1)$

	Gametes									
	$\frac{a_1b_1}{a_1b_1}$	$\frac{a_2b_1}{a_2b_1}$	$\frac{a_2b_2}{a_2b_2}$	$\frac{a_1b_2}{a_1b_2}$	$\frac{a_1b_1}{a_1b_2}$	$\frac{a_2b_1}{a_1b_1}$	$\frac{a_2b_2}{a_2b_1}$	$\frac{a_1b_2}{a_2b_2}$	$\frac{a_1b_1}{a_2b_2}$	$\frac{a_1b_2}{a_2b_1}$
u_1	.	.	.	.	$\frac{1}{3}$	$\frac{1}{3}$	.	.	.	$\frac{1}{3}$
u_2	$\frac{1}{3}$	$\frac{1}{3}$	.	$\frac{1}{3}$	.	.	.	.	.	.
u_3	$\frac{1}{6}$	.	.	.	$\frac{1}{6}$	$\frac{1}{6}$	.	.	$\frac{1}{6}$	$\frac{1}{3}$
u_4	$\frac{1}{6}$	$\frac{1}{6}$	.	.	$\frac{1}{2}$	.	$\frac{1}{6}$	.	.	.
u_5	$\frac{1}{3}$	$\frac{1}{6}$	.	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	.	$-\frac{1}{3}$
u_6	.	$-\frac{1}{6}$	.	$-\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	$-\frac{1}{6}$	$\frac{1}{6}$	.	$\frac{1}{3}$
u_7	.	$-\frac{1}{6}$	.	$-\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{2}$	$-\frac{1}{6}$	$\frac{1}{6}$	$-\frac{1}{3}$	1
u_8	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{1}{6}$	.	.	.	.	.	.

Double reduction and recombination frequencies are obtained from $\hat{U}$ as follows :

$$\hat{a}_a = \hat{u}_2+\hat{u}_4+\hat{u}_5-\hat{u}_6-\hat{u}_7+\hat{u}_8$$
$$\hat{a}_b = \hat{u}_2+\hat{u}_5+\hat{u}_8$$
$$\hat{y}_{ab} = \tfrac{1}{2}(\hat{u}_3+\hat{u}_4)+\hat{u}_5-\hat{u}_7+\hat{u}_8$$

By combining disomic gametic matrices for genotypes from both isomorphic sets, the deficiency matrix can be reduced to a row vector of rank one. This deficiency row vector is the same as that found for disomic gametic matrices for genotypes digenic at one locus and trigenic at the other.

6. NUMERICAL EXAMPLE

For the numerical example we shall consider tomato trisomic data presented by Lesley (1937). The data represent a portion of the disomic progeny from the cross $(d\text{PS})(\text{D}p\text{S})(\text{DP}s) \times (dps)_2$, where d = dwarf, p = peach, and s = compound inflorescence. These three loci have the order *d-p-s* in chromosome 2 of the tomato. We shall designate the chromosomal segments between loci "d" and "p" and between "p" and "s" as regions 1 and 2, respectively.

Two out of the five cultures exhibited poor germination and these yielded highly significant deviations from the expected 2 : 1 ratios for the genes P, p and S, s. Therefore, the present analysis is based on the three remaining cultures, which yield the following data :

$DPS = 26$	$Dps = 12$	$DPs = 37$	$DpS = 70$
$dps = 0$	$dPS = 57$	$dpS = 3$	$dPs = 9$

These data, although more satisfactory, are not wholly free from viability disturbances. The observed ratios are $D : d = 145 : 69$, $S : s = 156 : 58$, and $P : p = 129 : 85$. The corresponding χ^2's computed on a 2 : 1 theoretical ratio are ${}_{\text{D}}\chi^2_1 = 0{\cdot}11$, ${}_{\text{S}}\chi^2_1 = 3{\cdot}72$, and ${}_{\text{P}}\chi^2_1 = 3{\cdot}95$. The deficiency of non-peach (P) is the most surprising disturbance of the data.

Lesley (1937) gave the correct gametic matrix which in this paper is presented in table 11. By ignoring double crossing over and using various fractions of the total data (all five cultures) he estimated the recombination percentages to be 5·9 for region 1, 26·0 for region 2 and 30·0 for the region from "d" to "s" ignoring "p".

In the present analysis the estimation procedure outlined by Fisher (1947) is used to obtain maximum likelihood estimates of the five different modes of gamete formation and subsequently estimates of the various recombination values.

Fairly accurate initial trial values for mode frequencies are obtained by calculating the recombination value for each region separately. Thus, the recombination values 0·064,442 and 0·334,367 are computed for regions 1 and 2, respectively. The trial values for frequencies of modes 4 and 5 are obtained by halving the expected double crossover frequency, 0·021,547. Hence, the initial trial frequencies are $f_1 =$ 0·622,737, $f_2 = 0{\cdot}042{,}895$, $f_3 = 0{\cdot}312{,}820$ and $f_4 = f_5 = 0{\cdot}010{,}774$.

TABLE 24

Expected numbers for gametic classes associated with each mode

DPS	*DPs*	*DpS*	*Dps*	*dPS*	*dpS*	*dps*	*dPs*	Sum
■	44·421,906,00	44·421,906,00	■	44·421,906,00	0	0	0	133·265,718
3·059,843,34	1·529,921,67	1·529,921,67	■	■	1·529,921,67	0	1·529,921,67	9·179,530
22·314,493,34	0	11·157,246,67	11·157,246,67	11·157,246,67	0	0	11·157,246,67	66·943,480
0·768,545,34	0·384,272,67	0	0·384,272,67	0·384,272,67	0·384,272,67	0	0	2·305,636
0·768,545,34	0·384,272,67	0·384,272,67	0	0·384,272,67	0	0·384,272,67	0	2·305,636
26·911,427,36	46·720,373,01	57·493,347,01	11·541,519,34	56·347,698,01	1·914,194,34	0·384,272,67	12·687,168,34	214

These frequencies (see table 24) are used to obtain the expected numbers for the gametic classes associated with each mode.

The information matrix of θ_i is given in table 25, where θ_i are the introduced symmetrical variables defined by the relations

$$\frac{\partial m_k}{\partial \theta_i} = f_i \left(\frac{\partial m_k}{\partial f_i} - \sum_i f_i \frac{\partial m_k}{\partial f_i} \right),$$

(see Fisher, 1947).

TABLE 25

Information matrix

28·589,519,20	−3·079,692,46	−24·271,744,36	−0·767,494,23	−0·470,588,15
	1·452,242,78	1·307,959,21	0·308,197,59	0·011,292,94
		22·533,525,67	0·363,582,66	0·066,676,81
			0·092,825,32	0·002,888,67
				0·389,729,73

By omitting the fifth row and column the reduced matrix can be inverted to give the covariance matrix (table 26) for the remaining θ_i.

TABLE 26

Covariance matrix of θ_i

2·567,461,65	2·576,075,91	2·574,154,05	2·592,545,16
	4·918,510,36	2·571,647,06	−5·103,735,69
		2·628,289,30	2·450,544,05
			39·555,447,32

Finally the adjustments $\Delta f_1 = 0{\cdot}009{,}627$, $\Delta f_2 = -0{\cdot}008{,}798$, $\Delta f_3 = -0{\cdot}016{,}434$, $\Delta f_4 = 0{\cdot}026{,}559$, and $\Delta f_5 = -0{\cdot}010{,}954$ are obtained giving rise to new trial values $f_1 = 0{\cdot}632{,}364$, $f_2 = 0{\cdot}034{,}097$, $f_3 = 0{\cdot}296{,}386$, $f_4 = 0{\cdot}037{,}333$, $f_5 = -0{\cdot}000{,}180$.

TABLE 27

Covariance matrix for f_i

0·001,893,5	−0·000,215,7	−0·001,451,5	−0·000,228,5	0·000,002,3
	0·004,305,1	−0·000,684,8	−0·003,404,9	−0·000,000,3
		0·002,802,1	−0·000,667,9	0·000,002,2
			0·004,301,0	0·000,000,3
				−0·000,005,1

The process is repeated and another set of adjustments are obtained : $\Delta f_1 = 0{\cdot}002{,}605$, $\Delta f_2 = -0{\cdot}003{,}680$, $\Delta f_3 = 0{\cdot}000{,}705$, $\Delta f_4 = 0{\cdot}000{,}189$, and $\Delta f_5 = 0{\cdot}000{,}180$. These adjustments are regarded as sufficiently negligible. The final mode frequency estimates are then $f_1 = 0{\cdot}634{,}969$, $f_2 = 0{\cdot}030{,}417$, $f_3 = 0{\cdot}297{,}091$, $f_4 = 0{\cdot}037{,}523$, $f_5 = 0{\cdot}000{,}000$. The covariance matrix associated with these estimates is given in table 27.

The recombination fraction for region 1 is estimated as $\hat{y}_1 = f_2+f_4+f_5 = 0{\cdot}067{,}940 \pm 0{\cdot}042{,}323$ and for region 2, $\hat{y}_2 = f_3+f_4+f_5 = 0{\cdot}334{,}614 \pm 0{\cdot}075{,}942$. The estimated recombination fraction between the "*d*" and "*s*" loci, ignoring the "*p*" locus, is $\hat{y}_{ds} = f_2+f_3+f_5 = 0{\cdot}327{,}508 \pm 0{\cdot}075{,}738$. The fact that this fraction is smaller than $\hat{y}_2$

is due to the exceptionally large fraction of " progressive " double crossovers, *i.e.* $\hat{f}_4 = 0{\cdot}037{,}523$. The total frequency of double crossing over, $\hat{y}_{12} = \hat{f}_4 + \hat{f}_5 = 0{\cdot}037{,}523 \pm 0{\cdot}065{,}548$, is considerably larger than the expected frequency of double crossing over on the basis of no interference, *i.e.* $\hat{y}_1 \cdot \hat{y}_2 = 0{\cdot}022{,}734$. Apparently, all of the double crossovers involve only two chromosomes.

These data have been analysed merely to present a numerical example. Too much confidence should not be placed in these estimates because of viability disturbances and the large standard errors associated with the estimates.

7. DISCUSSION

The most extensive experimental studies involving linkage in trisomic inheritance are those of Bridges and Anderson (1925) and Redfield (1930 and 1932). The primary purpose of these studies was to compare recombination values for the same chromosomal region in diploid and triploid *Drosophila melanogaster*. By making such a comparison it was hoped to evaluate the possibility of sister-strand crossing over. Taken together, these experiments involved essentially the entire length of each of the three major chromosomes.

The experimental procedure consisted of crossing triploid females, which contained the appropriate mutants, with completely recessive diploid males. At the same time control matings between heterozygous diploid females and completely recessive males were made, and from these matings disomic linkage data involving the same loci were obtained.

Bridges and Anderson (1925) concentrated on the X-chromosome and identified 182 exceptional diploid female progeny. Thus, they evaluated disomic gametes from the triploid female parent and compared linkage data from these disomic gametes with the corresponding linkage data from the diploid control crosses. Redfield, on the other hand, concentrating on the second and third chromosomes, used monosomic gametes, from which recombination values were computed and compared with the corresponding estimates obtained from the diploid crosses.

It was generally found that recombination values estimated from diploid and triploid crosses were different for a given chromosomal region. However, these differences were quite variable and it was, therefore, impossible to make inferences regarding the phenomenon of sister-strand crossing over. Nevertheless, these studies have led to further investigations of factors influencing recombination frequencies (Schultz and Redfield, 1951).

What are the ideal conditions under which a comparison of crossing over in a trisome with that in a disome can be accurately made? Let us first consider the use of trisomics and then, briefly discuss the Drosophila triploid case. The problem common to both situations is to obtain unbiased recombination estimates.

With trisomics there are at least two possible sources of disturbance which need to be examined. These are (i) viability of mutants, and (ii) non-random disjunction of homologous chromosomes in the trisomic organism (see Rhoades, 1933, and Mather, 1933).

In order to detect and to some extent cope with these disturbance factors, it is necessary to obtain data from each of the three following sources :

(i) " balanced " disomic × disomic control crosses,
(ii) disomic progeny from trisomic × disomic crosses,
and (iii) trisomic progeny from trisomic × disomic crosses.

The experiments yielding such data should involve the same chromosomal segments, have, as nearly as possible, the same genetic background, and be conducted under identical conditions.

For the disomic × disomic control crosses, the use of " balanced " experiments allows an estimation of recombination values free from viability effects.

If the viability effects cause significant disturbances among either or both classes of progeny from the trisomic × disomic cross, the problem is difficult and it is not clear how unbiased recombination estimates can be obtained.

If the viability effects are negligible, then the comparison of recombination values computed from monosomic and disomic gametes permits an examination of the assumption of random disjunction of homologous chromosomes in the trisomic parent. Only if the two classes of gametes yield similar recombination values, but values which are significantly different from those derived from the control crosses, can it be concluded that there are factors other than those listed above operating to cause different recombination values at the disomic and trisomic levels.

When Drosophila triploids are used, the problem of ensuring unbiased recombination estimates is much more difficult, even if it can be assumed that viability effects are negligible. There are eight different chromosomal types of progeny resulting from the cross ♀ triploid × ♂ diploid. These are the result of the combination (distinctly non-random) of four egg types 1X+1A, 1X+2A, 2X+1A, and 2X+2A with the two types of sperms 1X+1A and 1Y+1A (where X and Y are the sex chromosomes and A represents a haploid set of autosomes). Absence of bias is ensured only when recombination values for a given region are similar for the eight different classes of progeny. However, it is impossible to examine this condition since certain types cannot be tested.

Thus, it would appear that if an accurate comparison of crossing over in a trisome with crossing over in a corresponding disome is to be made, it would be best to use *trisomics* in which random disjunction of homologous chromosomes occurs and to use mutants which have unimpaired viability.

A second problem pertains to the kind of trisomic genotype which is used for a study of linkage. The ideal is one which is trigenic at each locus. However, this genotype can seldom be obtained. Bridges and Anderson (1925) obtained an essentially equivalent genotype using only two alleles per locus. This was accomplished by marking the ends of the chromosomal regions by two very closely linked loci. In this way they were able to include four contiguous regions in the X-chromosome of *Drosophila melanogaster* from y = yellow (0·0) to B = bar (57·0). Using the same technique in one experiment, Redfield (1930) included five contiguous regions on the third chromosome of *Drosophila melanogaster* from *se* = sepia (26·0) to M = minute (101). In these cases the estimation procedure is simple and efficient in the sense that all data are utilised.

More often the experimenter will necessarily use trisomic genotypes which are digenic at all loci and in which the above procedure cannot be utilised. Such was the case in certain of Redfield's studies of the third chromosome and in her studies of the second chromosome. Digenic genotypes were also used in maize experiments (Rhoades, 1933) and in tomato experiments (Lesley, 1937). In all of these studies approximate estimation procedures were applied to data resulting from the monosomic gametes of the trisomic organism. Redfield computed recombination values for each region separately by multiplying the apparent recombination frequency by $\frac{3}{2}$. No attempt was made to estimate multiple crossover frequencies. The constant $\frac{3}{2}$ is the appropriate constant to use for this approximation, but the estimating procedure does not utilise all of the available information and is not the maximum likelihood estimate.

To consider the appropriate estimation procedure for obtaining the maximum amount of information from crosses involving trisomic genotypes which are digenic at all loci let us assume: (i) there are l loci involved, $l>2$, (ii) the cross trisomic $\times$ disomic (completely recessive) is made, and (iii) linkage data from both disomic and trisomic progeny are obtained. Maximum likelihood estimates can be computed by a procedure outlined by Fisher (1937). However, as pointed out earlier, computational limits are imposed by the dimensions of the information matrix. Hence analyses must be made on genotypes involving groups of genes in which the size of the group depends to some extent on the type of progeny. Thus, for disomic progeny, data can be analysed for genotypes involving up to four loci. Such analyses allow an examination of frequencies of various classes of multiple crossovers, and in addition, allow estimation of recombination values for any given pair of loci. For trisomic progeny, data can be analysed for genotypes involving two loci. Such analyses permit double reduction frequencies to be estimated for each locus, and permit estimation of recombination values for appropriate pairs of loci.

Thus, by utilising information obtained from both types of progeny

it may be possible to locate the relative position of the centromere with respect to the loci, to compare recombination estimates for any given pair of loci for both classes of progeny, and to estimate the frequencies of various kinds of multiple crossovers.

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THE USE OF SIRE AND DAM FAMILY AVERAGES IN INCREASING THE EFFICIENCY OF SELECTIVE BREEDING UNDER A HIERARCHICAL MATING SYSTEM

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1. INTRODUCTION

THE relative merits of individual selection and family selection in breeding for traits of incomplete heritability were first discussed by Lush (1947) and, more recently, with particular reference to poultry breeding, by Lerner (1950). The general conclusion is that for traits of low heritability, selection of complete families of full or half-sibs without regard to individual performance is more efficient than selection on the basis of individual phenotypes. For higher values of heritability the situation may be reversed, but in all cases maximum efficiency can be obtained by selection on the basis of a combination of family average and individual record.

In poultry and other animal populations the family classification is a hierarchical one, consisting of several sires each mated to a number of dams and each mating producing several offspring. Various schemes of family selection or combination selection are thus available :

(1) Selection of complete full-sib families disregarding any classification into half-sib families, *i.e.* selection on the basis of dam family average without regard to sire family average or individual merit.

(2) Selection on the basis of an index with particular weights attached to the full-sib family averages and to individual records.

(3) Selection of complete sire families alone, each sire family consisting of a mixture of full- and half-sibs.

(4) Selection on the basis of an index with particular weights attached to sire family averages, dam family averages, and individual records.

The first and second of these systems are discussed in detail by Lerner (1950, ch. XI) but neither of the others have been investigated, the nearest approach being the comparisons by Lerner and Lush of the relative merits of mass selection and half-sib family selection. These comparisons, however, neglect the full-sib relationship which exists between many pairs of a half-sib group in the above hierarchy.

It is evident that the first three of the above systems are particular

cases of the fourth—systems (1) and (2), for example, correspond to cases where zero weight is attached to the sire average—and hence that the efficiency of system (4) must always be greater than or equal to that of the others. One object of this investigation was thus to assess the optimum weighting factors applicable under system (4) and the increase in efficiency of selection attainable thereby. Secondly, it was judged of major importance to assess the relative efficiency of system (3)—selection of sire families alone, particularly in view of the drastic reductions in labour of individual recording which the system may permit.

System (3) may in fact be regarded as a modified " Hagedoorn Nucleus System ". This system of poultry breeding (Hagedoorn and Sykes, 1953) involves the selection of complete sire families without individual recording, followed by brother × sister or half brother × sister mating within the selected groups. Whilst the well-known deterioration of most production traits under inbreeding, coupled with the lowered selection differential available, throws serious doubts on the eventual outcome, it is shown here that the system of family selection is basically sound and can readily be adapted to a non-inbreeding scheme (paradoxically it must be noted that the theoretical efficiency of the method rests on the concept of heritability, summarily dismissed by Hagedoorn as unscientific). Adaptation of the method to a non-inbreeding scheme appears in fact to have been carried out by Sykes (1953), who obtained quite marked increases in egg production over a short test of three years (Hagedoorn and Sykes, p. 202). Likewise, Hagedoorn (pp. 65-66) claims similar success in the breeding of Khaki Campbell ducks by Jansen, but from the discussion by Hutt (1952) it seems clear that emphasis has been placed on the selection of progeny tested males, and also that individual recording of females has been the rule.

It would be premature to advocate without reservation Hagedoorn's recommendation that individual recording can be completely dropped in favour of recording families housed as units. Environmental effects peculiar to pens or units may preclude the recognition of true genetic differences, although in poultry there is some evidence (Dudley and Read, 1949 ; Hale, 1952) that uncontrollable variations between pens treated alike will be unimportant in this respect. However, even with individual recording under randomised environmental conditions it is important to note the value of the sire family average in a selection programme, since for traits of low heritability the greatest weight is to be attached to this parameter. Likewise, even with individuals housed at random, system (3) affords a much simplified system of recording, identification of, say, egg production being necessary only on a family basis with coloured leg bands being sufficient to identify the family to which an individual belongs. Such recording could be supplemented by individual information either from recording or handling as available.

2. THEORY

(i) *Basic concepts*

The principles of family and individual selection originate in the statistical studies of Fisher (1918) and Wright (1921), placed on an applied basis by Lush (1945, 1947) with particular emphasis on Wright's concept of heritability.

The simplest assumption in dealing with the inheritance of quantitative characteristics in this context is that phenotypic differences arise from the joint effect of independent and additive differences in genotype and individual environment. If environmental factors operate at random the phenotypic variance σ^2_P may be expressed as the sum of two parts, σ^2_G and σ^2_E, reflecting respectively the variance due to these agencies. Heritability, or the proportion of variance due to genetic differences, may then be expressed as $h^2 = \frac{\sigma^2_G}{\sigma^2_P}$.

The genetic value of an individual may be defined as the phenotypic value attained if subject to the average environment of the population, differences between genetic and actual phenotypic values being due to individual vagaries of environment. For prediction of gains from selection an estimate is required of the genetic value of individuals or groups selected on the phenotypic level. The population regression coefficient of genotype on phenotype is

$$\frac{\text{cov}(G, P)}{\sigma^2_P} = \frac{\text{cov}(G, G+E)}{\sigma^2_P} = \frac{\sigma^2_G}{\sigma^2_P} = h^2$$

(where G, P, E represent deviations from means due to each agency). Thus, individuals whose phenotypic mean differs from that of the parental population by an amount I have an expected genetic deviation of Ih^2, this quantity representing the expected deviation of the mean of their offspring from that of the parental population, or the expected gain from individual selection, if the same selection pressure I is applied to both male and female parents. If only females are selected the gain is $\frac{1}{2}Ih^2$ where I is measured in females only. The accuracy of such prediction in an individual sample or flock is, of course, higher the larger the sample ; otherwise for small samples it can only be regarded with confidence as the average gain expected from repeated samplings and selections.

Heritability can be defined either on an individual or on a family basis. In the latter case h^2_F is the regression of family genetic mean on phenotypic mean and, since the individual effects of environment cancel out in the family mean, is greater than h^2. The gain from selection of complete families is $I_F h^2_F$ where I_F, the selection differential, represents the deviation of the mean of selected families from the population mean. Since, except for certain limitations discussed later, a fixed proportion of the population is required for replacement whatever the criterion of selection, a more convenient nomenclature

is to express the expected gains as gains per unit standard deviation in the selection differential. Thus, if σ_P is the phenotypic standard deviation of individuals, σ_F that of families, the gains from individual and family selection are $\Delta G = h^2\sigma_P$ and $\Delta G_F = h^2_F\sigma_F$. The lowered selection differential corresponding to the lowered variability of family averages counteracts the tendency for the increased heritability of family averages to enhance efficiency of selection.

The above examples are particular cases of the rule that where selection is based on an index I the gain in character P (again per unit standard deviation in the selection differential) is $\Delta G_I = R_{IG}\sigma_G$ where R_{IG} is the correlation between genotype and criterion of selection (*cf.* Lush, 1947, p. 247 ; Lerner, 1950, p. 164). For selection on phenotypic values of individuals $R_{IG} = h$ giving $\Delta G = h\sigma_G = h^2\sigma_P$.

The present analysis is referred primarily to poultry populations where the main interest is centred on the production characteristics of a population of females. The efficiency of individual selection is taken as a basis for comparing the merits of different systems and since no criterion of individual merit may be available for selection of males the present comparisons are based throughout on the selection of females only. The principles of family selection may be readily extended to the selection of male parents on the performance of their full- and half-sisters, but to avoid unnecessary elaboration only the basic points will be mentioned here.

For selection of females a gain of 10 eggs from individual selection may be magnified to 15 eggs when family average is the criterion of selection, whilst if equally efficient selection of males is carried out the latter figure will be doubled. Various complicating factors, however, will be introduced. Firstly, if family merit is the criterion, the accuracy of identifying male genotypes will be less than for females, since only in the latter case are the individual phenotypes included in the family average. Secondly, the actual gains from male selection depend on the number of males which can be retained for future breeding and their distribution by families. If small numbers are available, the choice may be restricted to families other than those providing the superior females, and the efficiency of selection reduced. Conversely, if large family groups of males are available the relatively small number necessary to replace the flock may be obtained from a more restricted group of families than that involved in the selection of females, the efficiency of male selection thereby being increased. Assuming, however, the efficiency of selection to be the same for both sexes the following results for family selection represent only half the possible gains.

For combination selection the further gain to be expected from selection of males will be offset by the fact that individual merit cannot be included in the index of selection. These points will be dealt with more adequately in subsequent studies ; for the present it is sufficient to illustrate the relative merits of the systems for female

selection alone. Throughout the paper gains will thus be referred to as $\frac{1}{2}Ih^2$ instead of the customary Ih^2 when the same selection pressure applies to both parents.

(ii) *Efficiency of family selection on systems* (1) *and* (3)

It is assumed in what follows that selection is from a population of *s.d.n.* offspring originating from s sires each mated to d dams and each mating producing n offspring. The distribution of variance of offspring is then of the form in table 1, the model being that commonly used in estimation of heritability.

Q, D and S are respectively the variance components associated with full-sibs, true means of dam families within sire groups, and true

TABLE 1

Analysis of variance model for estimation of heritability

Source of variation	Degrees of freedom	Expected mean square
Between sires . . .	$s-1$	$Q+nD+ndS$
Between dams within sires .	$s(d-1)$	$Q+nD$
Between full-sibs . . .	$ds(n-1)$	Q

means of sire groups. It is readily shown (*e.g.* Comstock and Robinson, 1948) that for random mating and additive autosomal inheritance, values of these components are

$$Q = \tfrac{1}{2}\sigma^2_G + \sigma^2_E, \quad D = S = \tfrac{1}{4}\sigma^2_G.$$

Heritability in the infinite random bred population may then be defined as

$$h^2 = \frac{4S}{Q+D+S} = \frac{4D}{Q+D+S} = \frac{2(D+S)}{Q+D+S}$$

and may be estimated from sample values of components derived from actual mean squares. Alternatively the above ratios can be regarded as intra-class correlation coefficients, $S/(Q+D+S)$ for example representing the correction between half-sibs of $\frac{1}{4}h^2$.

Whilst the above model provides a valid estimate of heritability in the infinite random bred population it should be pointed out that for small samples a correction is, strictly speaking, necessary to the usual prediction of gain as $\frac{1}{2}Ih^2$. The closest estimate of gain is actually $\frac{1}{2}Ih^2_c$ where h^2_c is the regression of genotype on phenotype in the population from which the parents are selected. The expectation of this regression is G/P where G and P are the expected genetic and phenotypic variances (as the means of repeated sampling) of the

s.d.n. offspring. Pooling of mean squares in table 1 gives

$$P = Q + \frac{n(sd-1)D}{sdn-1} + \frac{nd(s-1)S}{sdn-1}$$

$$= \sigma^2_E + \sigma^2_G\left\{\frac{1}{2} + \frac{n}{4}\left(\frac{sd-1}{sdn-1}\right) + \frac{nd}{4}\left(\frac{s-1}{sdn-1}\right)\right\}$$

and $G = P - \sigma^2_E$.

The expected gain is thus $\frac{1}{2}(IG/P)$. The utility of the correction for small samples is seen by assigning particular values to s, d, and n. Case $s = d = 1$ corresponds to selection from a single randomly chosen full-sib family giving, as required,

$$P = \tfrac{1}{2}\sigma^2_G + \sigma^2_E,\ G = \tfrac{1}{2}\sigma^2_G \qquad \text{(see also Section 2.iii).}$$

Likewise $s = n = 1$ corresponds to selection from a single randomly chosen sire family where none of the half-sib progeny are full-sibs. Here

$$P = \tfrac{1}{2}\sigma^2_G + \sigma^2_E,\quad G = \tfrac{3}{4}\sigma^2_G.$$

For large or even moderately sized populations the correction is small. It is greatest when s and d are small with n large, when the full-sib relationship of individuals becomes important. For fixed d and n its importance declines as s increases. It is assumed, therefore, in what follows, that s is sufficiently large for the expectation of improvement to be taken as $\frac{1}{2}Ih^2$ or $\frac{1}{2}h^2\sigma_P$.

(a) *Selection of full-sib families.* For full-sib families of size n the expected phenotypic variance of the means of *unrelated* families is

$$P = \frac{Q}{n} + S + D = \sigma^2_G\,\frac{(n+1)}{2n} + \frac{\sigma^2_E}{n}$$

and genetic variance $G = \sigma^2_G\,\dfrac{(n+1)}{2n}$.

The expected gain $\Delta G_F = \frac{1}{2}h^2_F\sigma_F = \frac{1}{2}(G/p\frac{1}{2})$ readily reduces to

$$\frac{\frac{1}{2}h^2\sigma_P(n+1)}{\sqrt{2n\{2+(n-1)h^2\}}} \quad . \quad . \quad . \quad . \quad . \quad \text{(A)}$$

and the efficiency of selection relative to individual selection is

$$\frac{n+1}{\sqrt{2n\{2+(n-1)h^2\}}} \quad . \quad . \quad . \quad . \quad . \quad \text{(B)}$$

For the type of population involved here a correction is again necessary when s is small because of the half-sib relationship existing between some families. The expected " between family " mean square (pooling of sires and dams within sires) is

$$Q + nD + \frac{nd(s-1)}{sd-1}\,S,$$

the expected phenotypic variance of family means being

$$P = \frac{Q}{n} + D + \frac{d(s-1)}{sd-1} S$$
$$= \sigma^2_G\left\{\frac{1}{2n} + \frac{1}{4} + \frac{(s-1)d}{4(sd-1)}\right\} + \frac{\sigma^2_E}{n}$$
$$\text{and } G = P - \frac{\sigma^2_E}{n}.$$

Here again the correction is unimportant when, for fixed n and d, s is sufficiently large. Its importance is greatest when s and n are small and d is large, when the half-sib relationship of full-sib families assumes importance. As before, s is henceforward assumed to be sufficiently large for the efficiency of full-sib selection to be represented by (B), bearing in mind that it overestimates the efficiency for small samples.

(b) *Selection of sire families (half- and full-sibs).* Here the family size is dn, giving

$$P = \frac{Q}{dn} + \frac{D}{d} + S$$
$$= \sigma^2_G\left\{\frac{1}{2dn} + \frac{1}{4d} + \frac{1}{4}\right\} + \frac{\sigma^2_E}{nd},$$

G being represented by the first term. Expectation of improvement comes to

$$\Delta G_F = \frac{\frac{1}{2}h^2\sigma_P\{2+n(1+d)\}}{\sqrt{4dn[4+\{n(1+d)-2\}h^2]}} \quad . \quad . \quad . \quad (C)$$

and the efficiency relative to individual selection is

$$\frac{2+n(1+d)}{\sqrt{4dn[4+\{n(1+d)-2\}h^2]}} \quad . \quad . \quad . \quad (D)$$

An alternative derivation is to use the fact that

$$\Delta G = \frac{\{1+(n-1)r_G\}\frac{1}{2}h^2\sigma_P}{\sqrt{n\{1+(n-1)r_Gh^2\}}} \quad . \quad . \quad . \quad . \quad (E)$$

(Lush, 1948, p. 248)

where r_G is the genetic correlation between members of a family of size n. The average correlation between members of a sire family may be derived as

$$\bar{r}_G = \frac{1}{4}\left\{\frac{n(d+1)-2}{nd-1}\right\}$$

by taking the correlation of full-sibs as $\frac{1}{2}$ and that of half-sibs as $\frac{1}{4}$. (This is the value of $\bar{r}_G$ applied erroneously by Garber and Godbey (1952) to correction of heritability estimates). Substitution of $\bar{r}_G$ for r_G and nd for n in formula (E) gives formula (C).

Putting $n = 1$ in (C) gives

$$\Delta G_F = \frac{\frac{1}{2}h^2\sigma_P(d+3)}{\sqrt{4d\{4+(d-1)h^2\}}}$$

which may be readily deduced from first principles as the gain from selection of half-sib families, none of which are full-sibs.

It must be stressed that these relative gains will be approached with accuracy only for large populations. For small populations they correspond roughly to the average response from a series of selections but a further complication is introduced (particularly in sire family selection) by the reduction in selection differential caused by proportional selection from a small number of families. For an infinite population of families the selection differential for a selected

TABLE 2

Selection differentials and efficiency of selection for family selection when 20 per cent. of the families are required for replacement

Number of families	Number of families selected	Selection differential (I/σ_F) for 20 per cent. retained	Efficiency of selection relative to infinite population
5	1	1·16	0·83
10	2	1·27	0·91
20	4	1·33	0·95
30	6	1·35	0·96
40	8	1·36	0·97
50	10	1·37	0·98
Infinite	20 per cent.	1·40	1·00

proportion of 20 per cent. of the population is $1{\cdot}40\sigma_F$. For smaller groups of families the differential is reduced, 2 out of 10 families giving an effective coefficient of only $1{\cdot}27\sigma_F$. This is derived from table XX in Fisher and Yates' (1948) tables which gives, for ranked data, the average deviation of the r^{th} largest of samples of n observations from a normal distribution. Nordskog and Wyatt (1952) give the appropriate selection differentials per unit standard deviation for a replacement fraction of 20 per cent. These are given in table 2, along with the corresponding efficiency of selection relative to that for an infinite population. Theoretically the correction is applicable to all types of selection—individual, full-sib and sire family, but its importance will be negligible in the first case and most serious in the third.

A statistical limitation on family size is described by Lerner (p. 278) for the case of full-sib selection. The foregoing formulæ assume that the proportion of the flock kept for replacement is the same for both individual and family selection. For full-sib selection this is only true if the number of individuals per family is less than the number of families. As an example, in a flock of 900 in 90 families of 10 each, 90 individuals or 10 per cent. of the flock are required for replacement with the same family size. With full-sib selection,

9 families, again 10 per cent. of the flock, are required. Again, for family size of 30, one family or 3·3 per cent. of the population must be selected and 3·3 per cent. with individual selection. However, if family size is 45, only 20 individuals or 2·2 per cent. are required on individual selection, whereas the one full-sib family still selected on the family basis now comprises 5 per cent. of the population. Even if only a random sample of the selected family is taken for replacement, the reduction in selection differential and genetic gain is a necessary consequence. The limiting case for accuracy of the foregoing comparisons is that at least one (and an integral number) of sire families should be required for replacement. In sire family selection this imposes the restriction that the number of sire families should not be less than the number in a *full-sib* family. Thus, if the latter is 10, 10 per cent. are required for selection of individuals ; selection of a complete sire family, size *nd*, will still involve the same proportion if the population size is 10*nd*, *i.e.* there are at least 10 sire families.

Finally, the question of selecting integral numbers of sire families may assume some importance in small populations although not necessarily because of population size *per se*. If, for example, 6 sires are each mated to 10 dams and each dam produces 4 offspring, the population size is 240, and 60 individuals or 25 per cent. are required for replacement. In sire family selection the effective proportion of families selected, each containing 40 individuals, is 2 out of 6 or $33\frac{1}{3}$ per cent., with a corresponding reduction in efficiency. On the other hand, this difficulty would not arise if the number of sire families were any multiple of 4 since then 25 per cent. of the families would always be selected.

The errors introduced in these respects become unimportant for large values of *s* as do the corrections to individual and full-sib selection described earlier. Under such circumstances the relative efficiencies of full-sib and sire family selection are given by (B) and (D) as

$$\frac{n+1}{\sqrt{2n\{2+(n-1)h^2\}}} \quad \text{and} \quad \frac{2+n(1+d)}{\sqrt{4dn[4+\{n(1+d)-2\}h^2]}}$$

(c) *Comparison of gains.* Probably the most useful measure of improvement in egg production of poultry is the production index or egg production (individual or average) of all birds housed, regardless of mortality. The number of estimates of its heritability are very limited, though for the University of California flock an estimate in the region of 0·05 appears to be reliable (Dempster and Lerner, 1947 ; Lerner and Hazel, 1947 ; Lerner, 1950, pp. 125-128). King and Henderson (1954) obtained an estimate in the region of 0·20, but this increase was due to a statistical correction for date of hatch, heritability being estimated on a within-hatch basis on the assumption that no interactive effects with date of hatch are present.

Viability, closely associated with the production index, is also of low heritability. Lush, Lamoreux and Hazel (1948) obtained an

estimate of 0·083 for adult mortality whilst Robertson and Lerner (1949) reported a figure of 0·089. For mortality from lymphomatosis the comparable estimates were 0·059 and 0·048. A value of 0·026 was found by Robertson and Lerner for mortality from reproductive disorders. Hale (1954) found a heritability of 0·07 for chick viability, the correspondence of these estimates from various sources suggesting that values less than 0·10 may be the general rule.

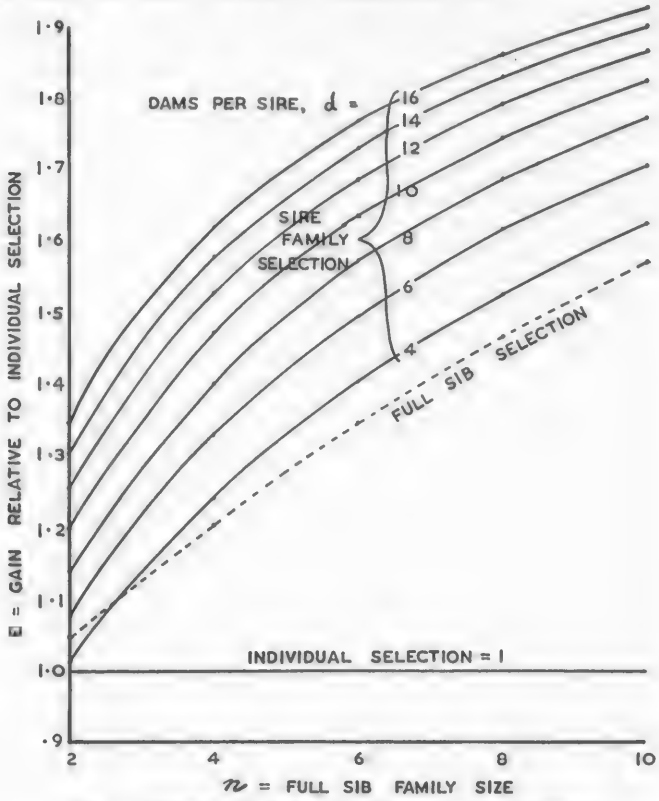

FIG. 1.—Efficiencies of selection of full-sib families and sire families relative to that of individual selection ($h^2 = 0{\cdot}05$).

Lerner (1950. p. 125) gives a list of other production characteristics —sexual maturity, winter pause, rate, persistency, egg size and survivors' production—all with heritability less than 0·125. As evident from a table of estimates in Jull (1952, p. 368) some of these must be regarded as under-estimates, but it is evident that particular attention should be paid to the applicability of formulæ (B) and (D) for heritabilities in the region of, say, zero to 0·20.

Figs. 1 to 3 show the relative efficiency of full-sib and sire family

selection for $h^2 = 0{\cdot}05$, $0{\cdot}10$, $0{\cdot}20$. The abscissæ represent n, the number of offspring per full-sib family, and the ordinates E, the efficiency of selection relative to individual selection. The relative

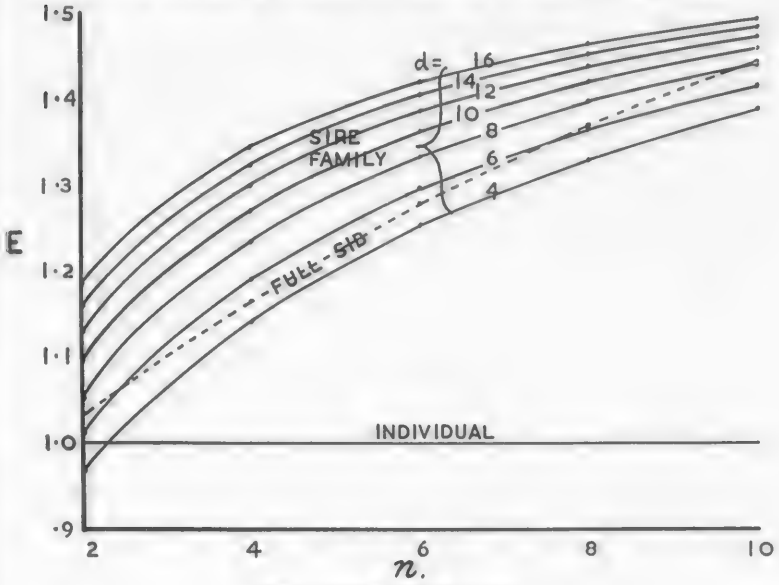

FIG. 2.—As fig. 1 for $h^2 = 0{\cdot}10$.

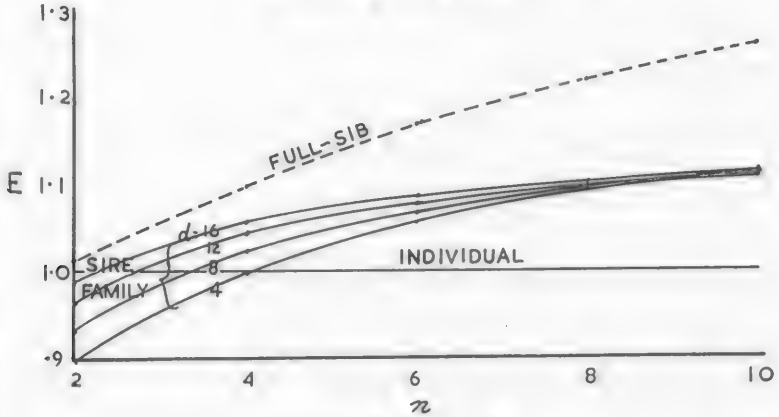

FIG. 3.—As fig. 1 for $h^2 = 0{\cdot}20$.

increase in efficiency of sire family selection over individual selection or full-sib selection is strikingly apparent for the lowest value of heritability of 0·05, its advantage declining with increase of the latter, until, at $h^2 = 0{\cdot}20$, the method is inferior to full-sib selection for all

values of n and d appropriate to these graphs. However, as shown later, even at these higher levels the weight to be attached to the sire family average in a combined index is still high.

At $h^2 = 0{\cdot}05$ " sire selection " is better than individual selection for all cases plotted and is better than full-sib selection except for the isolated case $d = 4$, $n = 2$. For $h^2 = 0{\cdot}10$ sire selection is better than individual selection except for $d = 4$, $n = 2$. If $d \geqslant 6$ it is again on the whole more efficient than full-sib selection, the exceptional cases being $d = 6$, $n < 3$ or $n > 8$; $d = 8$, $n \geqslant 10$. It is, however, apparent that the relative advantage declines rapidly with increasing h^2, as does the advantage of large values of d. At $h^2 = 0{\cdot}20$ the approach to coincidence of the curves for different values of d is apparent and sire selection is less efficient than full-sib selection; its overall advantage over individual selection, though small, is nevertheless still present except for $d = 4$, $n < 4$; $d = 8$, $n < 4$; $d = 12$, $n = 2$; $d = 16$, $n = 2$.

Several points regarding these graphs require to be emphasised. From fig. 1, if $n = 2$ the gain from full-sib selection relative to individual selection is 1·05, an increase of 5 per cent. The gain from sire family selection relative to individual selection for $n = 2$, $d = 16$ is 1·35, an increase of 35 per cent. or 7 times the relative gain from full-sib selection (actually 7·19). At $n = 10$ the appropriate value has been reduced from 7·19 to 1·63. This is illustrated in fig. 4 (deducible from fig. 1) where the ordinate G represents the ratio of the percentage gain from sire selection over individual selection to the percentage gain from sib selection over individual selection. In an average case $n = 6$, $d = 12$ the value of the ratio is approximately 2 (actually 1·98). This corresponds to the values from fig. 1 of 1·347 for the efficiency of full-sib selection and 1·688 for the efficiency of sire family selection.

The above, however, is not to be interpreted as a case for family selection with small rather than large full-sib families. The lower the number of offspring per breeding dam, the greater is the proportion of the flock necessary for replacement, with a consequent drop in absolute efficiency of individual or family selection. Case $n = 10$ corresponds to a percentage of 10 necessary for replacement; for a standardised normal distribution the normal deviate is $x = 1{\cdot}2816$, the ordinate at this point $z = 0{\cdot}1753$ and the mean of the selected group is $z/q = 1{\cdot}753$ (q = percentage selected). If $n = 2$ the mean of the 50 per cent. selected is reduced to 0·798 with a corresponding reduction of gain from individual selection. In this respect the most useful graphs are those of fig. 5 where the gain from individual selection with $n = 2$ is taken as unity and heritability is 0·05. The lower curve represents the gain from individual selection with different family sizes computed from statistical tables on the above basis, the second the gain from full-sib selection, and the higher curves the gains from sire selection for different values of d.

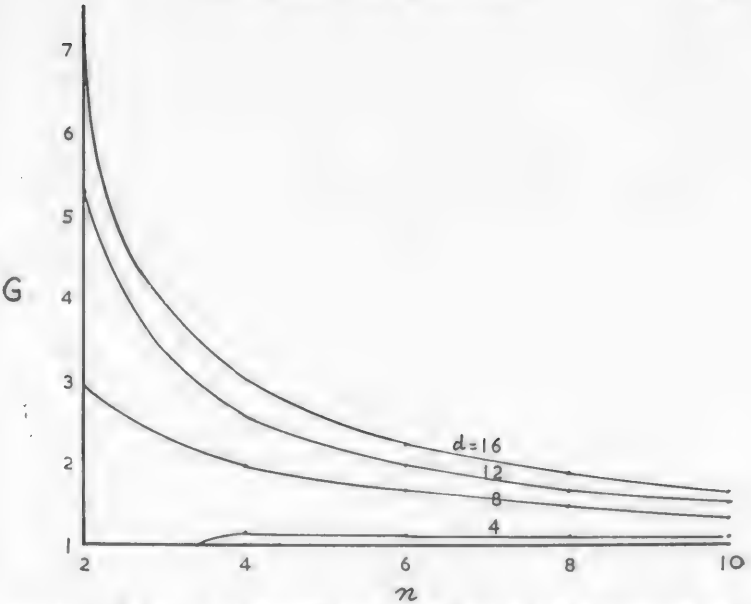

FIG. 4.—G, the ratio of the percentage gain from sire selection over individual selection to the percentage gain from sib selection over individual selection ($h^2 = 0{\cdot}05$).

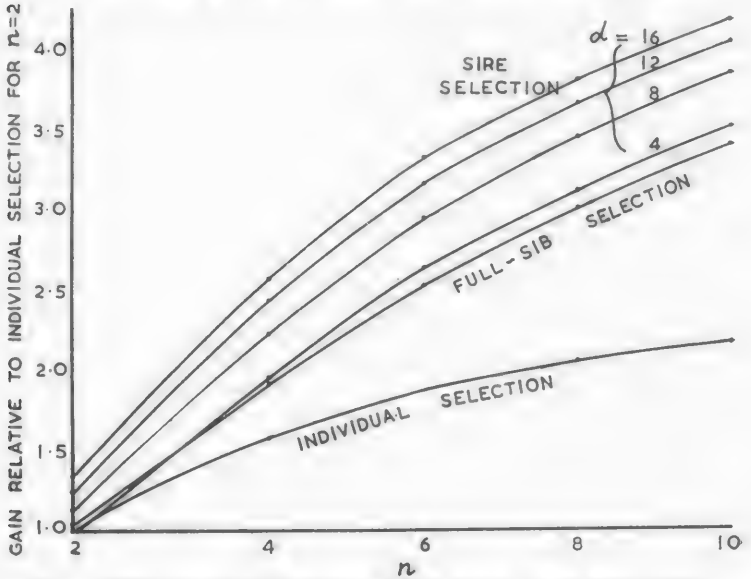

FIG. 5.—Efficiencies of individual selection, full-sib selection and sire family selection, relative to that of individual selection for $n = 2$ ($h^2 = 0{\cdot}05$).

For most of the cases illustrated maximum increases are obtained with maximum d so that in so far as flock size can be increased *by increasing* d so is the efficiency of sire selection (relative and absolute) increased. In general d will be determined by the mating limitations of the male parent and also by n, since the latter determines the total number of females necessary for replacement. The obvious and commonsense interpretation of the graphs is that (barring complications due to extension of the breeding season and the statistical limitation described previously) maximum gains from individual or family selection are obtained by increasing n. This determines sd, the number of selected females, whilst the greatest efficiency of sire selection is obtained by maximising d and minimising s. All effects are thus cumulative ; although the preceding results are referred to selection of females only, a minimal value of s will further correspond to a greater intensity of selection of males.

A further point so far as poultry are concerned is that complications introduced by date of hatch render inadvisable an indiscriminatory increase in family size. Lerner (1950, p. 276) discusses the problem in terms of the reduction of heritability expected by increase of environmental variability occasioned by extension of hatching season. On the other hand, Osborne (1954*a*) has described an *increase* in the heritability of age at maturity occasioned by such extension, genetic variability being only apparent between individuals subject to greater extremities of climatic variation. Likewise, interactions of genotype and environment for the same characteristic reported by Osborne (1952) have since been reported by Skaller and Sheldon (1955). In 4 out of 5 flocks investigated by the latter authors, interaction significant at the 10 per cent. or higher point was found between sire genotypes and date of hatch. Worthy of further investigation is the unsubstantiated claim by Skaller and Sheldon that "it is doubtful whether large errors would result from the application of present correction methods" (for linear changes in production characteristics occasioned by date of hatch). However, for short hatching season and values of n normally found in practice, say $n = 5$, the plotted values of efficiency may be regarded with confidence, the optimum value of n being a subject demanding further investigation.

Another error, whose importance is yet subject to investigation, will be introduced for characteristics involving mortality, where the number per family at the selection stage differs from the original number on which heritability estimates are based. Actually the error would appear to operate by underestimating the efficiency of family selection since this system will almost certainly (in traits such as egg production) involve selection of superior members of superior families, the system becoming effectively selection on a combination of family average and individual merit.

It is finally to be noted that the relative efficiencies are unaffected

by varying family size provided n and d are replaced by their average values calculated in the appropriate manner (*e.g.* Anderson and Bancroft, 1952 ; Osborne, 1954*b*). In general arithmetic averages should provide a sufficiently good approximation.

(iii) *Combination selection under system* (4)

Here it is required to estimate the genetic value G (estimate $\hat{G}$) of an individual in terms of phenotype P, dam family average F_D, and sire family average F_S. As a deviation from $\bar{G}$, the population mean,

$$\hat{G}-\bar{G} = (\hat{G}-G_D)+(G_D-G_S)+(G_S-\bar{G}),$$

the deviation being expressed as the sum of three independent parts :

(1) the deviation of estimated genetic value from the genetic mean of the dam family ;
(2) that of the dam family genetic mean from the sire family genetic mean ;
(3) that of the sire family genetic mean from the population genetic mean.

$(\hat{G}-G_D)$ may be expressed in the customary form for regression of genotype on phenotype

$$\hat{G}-G_D = h_1^2(P-F_D)$$

where h_1^2 is the effective heritability within full-sib families. Likewise

$$G_D-G_S = h_2^2(F_D-F_S)$$

where h_2^2 is the heritability of full-sib averages within sire families, and finally

$$G_S-\bar{G} = h_3^2(F_S-\bar{P})$$

where h_3^2 is the heritability of sire family averages (*cf.* Lush, 1947, p. 259, and Robertson, 1955).

Since $(P-F_D)$ is measured from the sample family mean the effective phenotypic variance within dam families (see table 1) is

$$\sigma_1^2 = \frac{n-1}{n} \cdot Q = \frac{n-1}{n} \left\{\tfrac{1}{2}\sigma^2_G+\sigma^2_E\right\}$$

and genetic variance $\sigma_1^2{}_G = \dfrac{n-1}{n} \cdot \tfrac{1}{2}\sigma^2_G$.

Thus $$h_1^2 = \frac{\sigma_1^2{}_G}{\sigma_1^2} = \frac{\tfrac{1}{2}\sigma^2_G}{\tfrac{1}{2}\sigma^2_G+\sigma^2_E} = \frac{h^2}{2-h^2}.$$

For means of dam families within sire families the phenotypic variance is

$$\sigma_2^2 = \frac{d-1}{d}\left\{\frac{Q}{n} + D\right\} = \frac{d-1}{d}\left\{\frac{\sigma^2_G}{2n} + \frac{\sigma^2_E}{n} + \frac{\sigma^2_G}{4}\right\}$$

and genetic variance $\sigma_2^2{}_G = \dfrac{d-1}{d}\left\{\dfrac{\sigma^2_G}{2n} + \dfrac{\sigma^2_G}{4}\right\}$,

giving $$h_2^2 = \frac{h^2(n+2)}{4+(n-2)h^2}.$$

$$\text{Similarly } h_3^2 = \frac{\left\{\frac{\sigma^2_G}{2nd} + \frac{\sigma^2_G}{4d} + \frac{\sigma^2_G}{4}\right\}}{\left\{\frac{\sigma^2_G}{2nd} + \frac{\sigma^2_E}{nd} + \frac{\sigma^2_G}{4d} + \frac{\sigma^2_G}{4}\right\}} = \frac{h^2\{n(1+d)+2\}}{4+\{n(1+d)-2\}h^2}.$$

Thus the weighted function for optimum estimation of genetic values and hence for efficiency of selection is

$$I = \hat{G}-\bar{G} = \frac{h^2}{2-h^2}(P-F_D) + \frac{h^2(n+2)}{4+(n-2)h^2}(F_D-F_S) + \frac{h^2\{n(1+d)+2\}}{4+\{n(1+d)-2\}h^2}(F_S-\bar{P}) \quad . \quad (F)$$

TABLE 3

Weighting coefficients for index of selection

$I = (P-\bar{P}) + b_2(F_D-\bar{P}) + b_3(F_S-\bar{P})$

d	$h^2 = 0{\cdot}05$				$h^2 = 0{\cdot}10$			
	$n = 2$	4	6	8	$n = 2$	4	6	8
	$b_2 = 0{\cdot}950$	1·854	2·714	3·534	$b_2 = 0{\cdot}900$	1·714	2·455	3·130
2	$b_3 = 1{\cdot}764$	3·213	4·411	5·406	$b_3 = 1{\cdot}555$	2·666	3·331	3·837
4	3·368	5·901	7·841	9·341	2·850	4·493	5·487	6·100
6	4·833	8·184	10·586	12·346	3·946	5·922	6·995	7·593
8	6·175	10·146	12·832	14·705	4·886	7·042	8·111	8·651
10	7·410	11·851	14·702	16·610	5·700	7·944	8·969	9·441
12	8·233	13·346	16·286	18·179	6·413	8·686	9·649	10·053
14	8·943	14·668	17·643	19·495	7·041	9·306	10·202	10·540
16	9·561	15·845	18·819	20·615	7·600	9·833	10·660	10·939
	$h^2 = 0{\cdot}020$				$h^2 = 0{\cdot}30$			
	$b_2 = 0{\cdot}800$	1·455	2·000	2·462	$b_2 = 0{\cdot}700$	1·217	1·615	1·931
2	$b_3 = 1{\cdot}200$	1·745	2·000	2·110	$b_3 = 0{\cdot}915$	1·183	1·248	1·238
4	2·057	2·756	3·000	3·056	1·488	1·761	1·772	1·705
6	2·700	3·415	3·600	3·593	1·879	2·105	2·060	1·950
8	3·200	3·879	4·000	3·938	2·164	2·332	2·242	2·101
10	3·600	4·235	4·286	4·180	2·380	2·493	2·367	2·203
12	3·930	4·488	4·500	4·358	2·550	2·614	2·459	2·277
14	4·200	4·699	4·666	4·495	2·687	2·708	2·529	2·333
16	4·431	4·871	4·800	4·603	2·800	2·783	2·585	2·377

Although statistical derivation from first principles is more complex, the practical application of this formula is simpler when all quantities are expressed as deviations from the population mean. Equation (F) may be adjusted to give

$$I = \frac{h^2}{2-h^2}(P-\bar{P}) + \frac{h^2}{2-h^2}\cdot\frac{2n(1-h^2)}{4+(n-2)h^2}(F_D-\bar{P}) + \frac{h^2 4nd(1-h^2)}{\{4+(n-2)h^2\}\{4+[n(1+d)-2]h^2\}}(F_S-\bar{P})$$

or, since only the relative weights are important in selection,

$$I = (P-\bar{P}) + \frac{2n(1-h^2)}{4+(n-2)h^2}(F_D-\bar{P}) + \frac{4nd(1-h^2)(2-h^2)}{\{4+(n-2)h^2\}\{4+[n(1+d)-2]h^2\}}(F_S-\bar{P}) \quad . \quad (G)$$

As a check, when $n = 1$ the individual value and full-sib average are identical and the case corresponds to combination selection on individual record and half-sib average. (G) reduces to

$$I = \frac{3(2-h^2)}{4-h^2}(P-\bar{P}) + \frac{4d(1-h^2)(2-h^2)}{(4-h^2)\{4+(d-1)h^2\}}(F_S-\bar{P})$$

where the ratio of coefficients is $4d(1-h^2)/3\{4+(d-1)h^2\}$. This may be independently obtained from the formula of Lush (1947, p. 257) as $d(r-t)/(1-r)\{1+(d-1)t\}$ where $r = \frac{1}{4}$ and $t = \frac{1}{4}h^2$.

Likewise if $d = 1$, F_D and F_S are synonymous, corresponding to full-sib selection with unrelated families,

$$I = (P-\bar{P}) + \frac{2n(1-h^2)}{2+(n-1)h^2}(F_D-\bar{P}).$$

The ratio $2n(1-h^2)/\{2+(n-1)h^2\}$ is similarly derived from Lush's formula and corresponds to the weight for family average in full-sib selection provided the half-sib relationship of families can be neglected.

Formula (G) is of course applicable for all values of heritability. As expected, the weight attached to F_S is large for low values of h^2 for which the superiority of sire family selection has been demonstrated. Regarding now P, F_S and F_D as deviations from means, the function, for $h^2 = 0{\cdot}05$, $n = 6$, $d = 10$, becomes $I = P+2{\cdot}714\ F_D+14{\cdot}702\ F_S$. In spite of the fact that with increasing h^2 sire family selection soon becomes less efficient than full-sib selection and eventually less efficient than individual selection, the major importance of the sire average when combined with individual values and full-sib averages extends to quite high values of h^2. At $h^2 = 0{\cdot}25$, $n = 6$, $d = 10$, $I = P+1{\cdot}80\ F_D+3{\cdot}15\ F_S$. Even at $h^2 = 0{\cdot}50$ the function, for $n = 2$ and $d = 16$, is $I = P+0{\cdot}50\ F_D+1{\cdot}20\ F_S$. However, as expected and as illustrated later, the relative gain in efficiency of the function becomes smaller with increased heritability and for poultry its main utility will be for values less, say, than 0·30. Table 3 gives the appropriate weights for n between 2 and 8, d between 2 and 16 and $h^2 = 0{\cdot}05$, 0·10, 0·20 and 0·30, whilst fig. 6 illustrates the variation in weighting factors with heritability for an average case $n = 6$.

One important restriction in the use of these weights is for varying family size. For the simpler single type family classification as discussed by Lerner and Lush, *e.g.* full-sib families of size n, different weights can be applied to families of varying size, the regressions being regarded as those obtained in hypothetical populations of family

size n_1, n_2 and so on. Each family is thus given weights appropriate to its own value of n. For the case discussed here the comparable situation is that each sire family may be assigned weights according to its value of d, but the results are derived on the assumption that n, within a sire group, is constant. It is doubtful that serious errors will arise by replacing n by its average value within a particular sire group unless marked variation is present, but the problem is subject to further investigation.

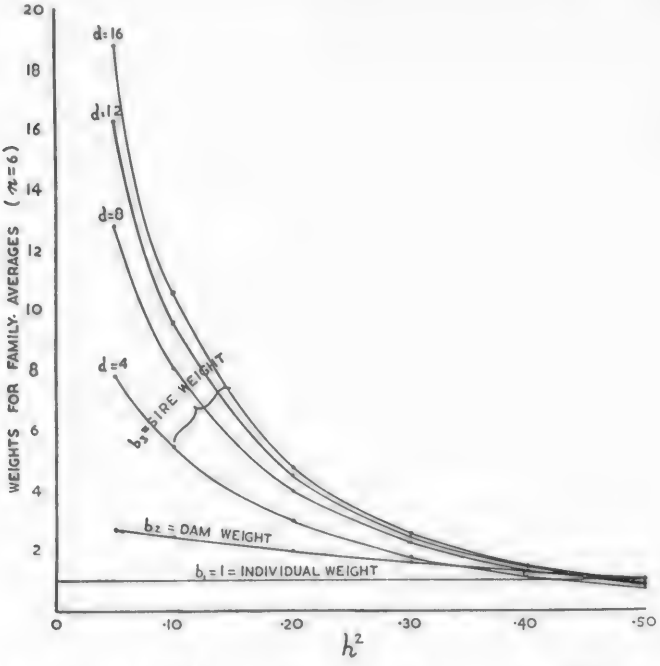

FIG. 6.—Weighting factors for individual performance ($b_1 = 1$), full-sib average (b_2), and sire family average (b_3), when the full-sib families are of size 6, $h^2 = 0$ to 0·50.

Relative efficiency of system (4). The gain from selection on an index I is $\Delta G_I = \frac{1}{2} R_{IG} h \sigma_P$ so that R_{IG}/h measures the efficiency relative to that of individual selection.

For multiple regression of genotype G on the mutually independent factors $(P-\bar{F}_D)$, $(\bar{F}_D-\bar{F}_S)$ and $(\bar{F}_S-\bar{P})$ the multiple correlation coefficient R is defined by

$$R^2 = b_1^2 \frac{\sigma_1^2}{\sigma_G^2} + b_2^2 \frac{\sigma_2^2}{\sigma_G^2} + b_3^2 \frac{\sigma_3^2}{\sigma_G^2}$$

where b_1, b_2 and b_3 are the regression coefficients on the three variables respectively and σ_1^2, σ_2^2, σ_3^2 are the variances of the three variables.

As in the previous section

$$b_1 = h_1^2 = \frac{h^2}{2-h^2}\ ;\ b_2 = h_2^2 = \frac{h^2(n+2)}{4+(n-2)h^2}\ ;$$

$$b_3 = h_3^2 = \frac{h^2\{n(1+d)+2\}}{4+\{n(1+d)-2\}h^2}\ ;$$

$$\sigma_1^2 = \frac{n-1}{n} \cdot \sigma_p^2 \frac{(2-h^2)}{2}\ ;\ \sigma_2^2 = \frac{d-1}{d} \cdot \sigma_p^2 \frac{\{4+(n-2)h^2\}}{4n}\ ;$$

$$\sigma_3^2 = \frac{s-1}{s} \cdot \sigma_p^2 \frac{\{4+[n(1+d)-2]h^2\}}{4dn}.$$

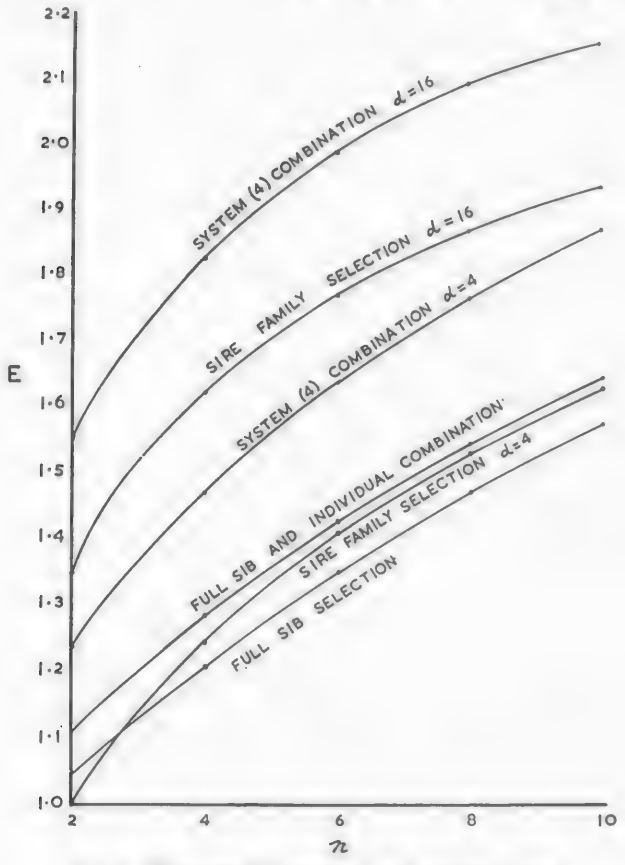

FIG. 7.—Efficiencies of all systems (1) to (4) relative to that of individual selection ($h^2 = 0{\cdot}05$).

Assuming, as before, the number of sires to be large, the factor $(s-1)/s$ can be ignored, giving on substitution and simplification, the efficiency

relative to individual selection as

$$\frac{R}{h} = \left[\frac{n-1}{n}\cdot\frac{1}{2(2-h^2)} + \frac{(d-1)}{d}\cdot\frac{(n+2)^2}{4n\{4+(n-2)h^2\}} + \frac{\{n(1+d)+2\}^2}{4dn\{4+[n(1+d)-2]h^2\}}\right]^{\frac{1}{2}} \quad . \quad . \quad \text{(H)}$$

As a check, if $d = 1$, (H) reduces to

$$\left[1 + \frac{(n-1)(1-h^2)^2}{(2-h^2)\{2+(n-1)h^2\}}\right]^{\frac{1}{2}}$$

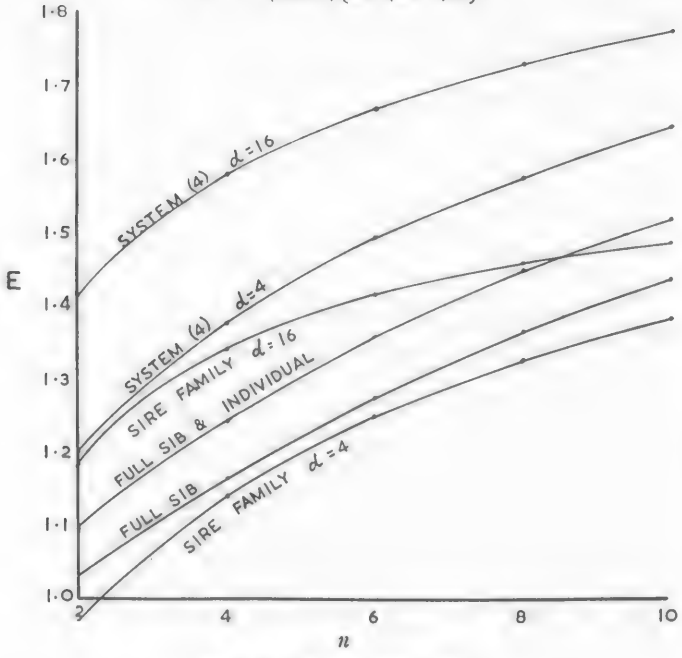

FIG. 8.—As fig. 9 for $h^2 = 0{\cdot}10$.

given by Lerner (p. 218) for combination selection on individual record and unrelated full-sib family averages. Similarly the correct result for combination of individuals and half-sib families may be obtained by putting $n = 1$.

Figs. 7 and 8 show the relative efficiencies of all systems (1) to (4) for $h^2 = 0{\cdot}05$ and $h^2 = 0{\cdot}10$ and the superiority of combination selection on system (4) over any of the others, particularly for large d, is evident. A typical example of the relative efficiencies for $n = 6$, $d = 16$, $h^2 = 0{\cdot}05$, taking the gain from individual selection as 10 units, is

(1) Full-sib selection : 13·5 ;

(2) Combination selection on full-sib average and individual record : 14·25 ;
(3) Sire family selection : 17·7 ;
(4) Combination selection on full-sib average, sire-family average and individual record : 19·8.

A striking feature is that whilst system (2) may give little advantage over system (1), the gain in efficiency of systems (3) and (4) over (2)

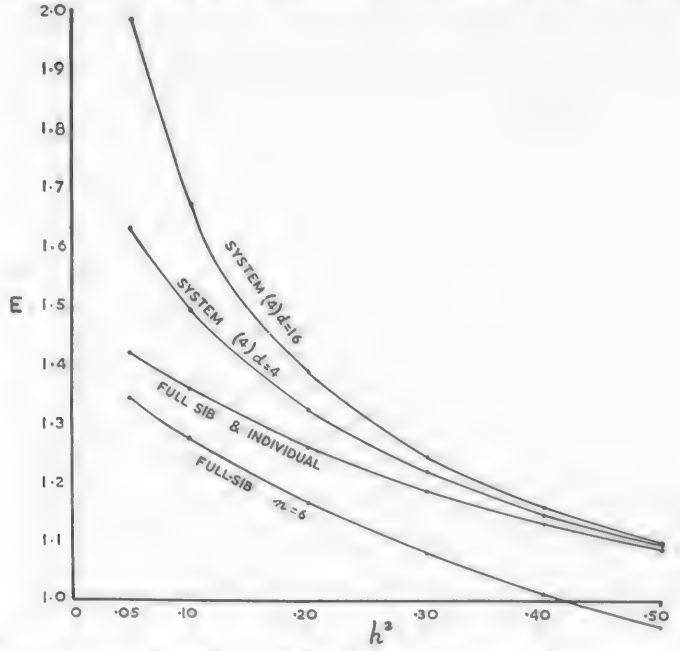

FIG. 9.—Efficiencies of systems (1), (2) and (4) relative to individual selection when full-sib families are of size 6, $h^2 = 0$ to 0·50.

may be quite considerable, and likewise the gain from (4) over the gain from (3).

Fig. 9 shows, for an average case $n = 6$, and all values of h^2 up to 0·50, the relative efficiency of systems (1), (2) and (4), the latter for $d = 4$ and $d = 16$. The advantage of system (4) over the others declines with increasing heritability, as does the advantage of each system over individual selection, but for the range applicable to many production traits, it is clear that worthwhile improvements may be obtained by judicious consideration of family average and individual record. In the important case where recording facilities are limited, something approaching maximum efficiency may be obtained by consideration of the sire family average alone.

3. SUMMARY

1. Systems of family selection or selection on a combination of family merit and individual merit are discussed for a population where, as in poultry, the family classification is a hierarchical one, consisting of offspring from several sires each mated to several dams. Four methods are compared with each other and with individual selection, the comparisons referring to selection of females in a population of females as in poultry :

(*a*) Selection of complete full-sib families disregarding individual merit or classification into half-sib families.

(*b*) Selection on the basis of an index with particular weights attached to full-sib family averages and to individual records.

(*c*) Selection of complete sire families, each family consisting of a mixture of full- and half-sibs.

(*d*) Selection on an index with particular weights attached to sire family averages, dam family averages, and individual records.

2. Systems (*a*) to (*c*) may be regarded as particular cases of (*d*) and it is axiomatic that the latter provides greatest efficiency. A general formula is derived giving appropriate weighting factors for all values of heritability and distributions of offspring by families, and these are tabulated for particular cases. For low values of heritability in particular, marked increases in efficiency are obtainable and even up to high values (subject to restrictions imposed by the distribution of offspring), greatest weight is to be attached to sire family average, less weight to dam family average, and least of all to individual performance.

3. For low values of heritability, system (*c*), selection of sire families alone, may be markedly more efficient than individual selection or family and combination selection on systems (*a*) and (*b*). This feature may prove of major importance in populations where trap-nesting or individual recording facilities are limited. Provided the intervention of environmental effects can be neglected (as some published evidence would indicate) selection for production traits with low heritabilities such as egg production may be based on sire family averages alone, each family being housed as a unit without individual recording.

4. Apart from complications introduced by extension of the breeding season maximum gains from systems (*c*) and (*d*) may be obtained by selecting the minimum number of breeding females necessary to replace the population, and maximising the number of dams mated to a given sire. Whilst these gains are referred to selection of females alone it is evident that all effects are cumulative since this procedure provides a higher possible selection pressure on male parents. Greater gains from selection, at least for low values of

heritability are possible in larger populations providing the difference in population size is due only to the number of dam families in a sire group.

5. Corrections are given for the orthodox expectation of gain from individual and family selection when small populations are involved.

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AN EFFECT OF GENE ARRANGEMENT ON THE RECOMBINATION FRACTION IN *DROSOPHILA MELANOGASTER*

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1. INTRODUCTION

This paper presents and analyses data for three linked loci situated at the distal end of the sex chromosome of *Drosophila*.

A systematic analysis was made possible by arrangement of the data in an orthogonal 4×4 Latin square. This arrangement allows factors such as viability and misclassification to be detected and removed so as to obtain accurate estimates of the recombination values. In the past, little use has been made of this design although some examples may be quoted (Anderson, 1921 ; Bridges and Morgan, 1916, 1919, 1923, and Wallace, 1947, 1957).

In the data presented in this paper significant differences of genotypic viability were found. After this viability effect had been allowed for, there was a significant variation in recombination according to the arrangement of the genes in the triple heterozygote. This may be explained as a position effect.

2. METHOD

The loci studied were scute (*sc*), cross-veinless (*cv*) and vermilion (*v*) situated at the distal end of the sex-chromosome. Scute is at 0, *cv* at 13·7, and *v* at 33·0 (Bridges and Brehme, 1944). One Canton-S wild type female was mated to *sc cv v* males and the resultant heterozygous females backcrossed to *sc cv v* males. The segregants from this mating were then intercrossed to obtain the four triply heterozygous females :—

$$\frac{sc\ cv\ v}{+\ +\ +}\ ,\ \frac{sc\ +\ -}{-\ cv\ v}\ ,\ \frac{sc\ cv\ +}{-\ +\ v}\ ,\ \frac{sc\ -\ v}{-\ cv\ +}$$

The members of any one of these four triply heterozygous genotypes were first cousins to the members of the other three. Within the triply heterozygous genotypes $\frac{sc\ cv\ v}{+\ +\ +}$, and $\frac{sc\ cv\ +}{+\ +\ v}$, all females were sibs but within the other two genotypes there were some first cousins. In the latter case a single mating did not supply enough triple heterozygotes and progeny from a second mating had to be used.

Twenty-five virgin females of each genotype were held until they were 48 hours old to avoid excessive fluctuations in recombination due to age differences (Schultz and Redfield, 1951). They were then mated separately to *sc cv v* males for 24 hours only, to prevent heterogeneity due to overcrowding. The entire experiment was carried out at 25° C. to avoid variations in recombination due to temperature differences (Plough, 1917). When the flies commenced to emerge, classification was carried out daily to minimise any losses after birth due to poor viability of any of the genotypes. Classifying the three mutant genes was easy and could be done

at emergence with the limitation that the wings had to be unfolded to classify crossveinless.

Four pairs of complementary genotypes are obtained in the progeny :—

sc cv v, + + +	E
+ *cv v*, *sc* + +	F
sc cv +, + + *v*	G
sc + *v*, + *cv* +	H

In relation to any one type of heterozygous female parent, the four pairs of complementary genotypes E, F, G, H, are obtained by four modes of gamete formation. The data are arranged as a 4×4 Latin square in which the columns represent the four modes of gamete formation, and the rows the four types of heterozygous female parents :—

Female parent	Type of gamete formation			
	No change	*sc* change	*v* change	*cv* change
sc cv v/+ + + .	E	F	G	H
+ *cv v*/*sc* + + .	F	E	H	G
sc cv +/+ + *v* . .	G	H	E	F
sc + *v*/+ *cv* + . .	H	G	F	E

TABLE 1

Observed data

Female parent	Type of gamete formation				
	No change	*sc* change	*v* change	*cv* change	
$\frac{sc\ cv\ v}{+\ +\ +}$	*sc cv v* 736 + + + 933	*sc* + + 140 + *cv v* 103	*sc cv* + 206 + + *v* 274	*sc* + *v* 6 + *cv* + 6	
	1669	243	480	12	2404
$\frac{+\ cv\ v}{sc\ +\ +}$	*sc* + + 888 + *cv v* 758	*sc cv v* 143 + + + 143	*sc* + *v* 264 + *cv* + 226	*sc cv* + 8 + + *v* 3	
	1646	286	490	11	2433
$\frac{sc\ cv\ +}{+\ +\ v}$	*sc cv* + 609 + + *v* 741	*sc* + *v* 162 + *cv* + 116	*sc cv v* 185 + + + 242	*sc* + + 5 + *cv v* 10	
	1350	278	427	15	2070
$\frac{sc\ +\ v}{+\ cv\ +}$	*sc* + *v* 825 + *cv* + 781	*sc cv* + 201 + + *v* 206	*sc* + + 251 + *cv v* 234	*sc cv v* 15 + + + 12	
	1606	407	485	27	2525
	6271	1214	1882	65	9432

3. THE EXPERIMENTAL DATA WITH ITS PRELIMINARY ANALYSIS

The data are summarised in the Latin square below (table 1). Each pair of complementary genotypes is split into its components, with the totals shown, these corresponding to the letters of the Latin square.

TABLE 2

Heterogeneity χ^2 values for replicates within each maternal genotype

Maternal genotype	No. of replicates	D.F.	χ^2	Probability
$\frac{sc\ cv\ v}{+\ +\ +}$	25	48	48·4478	0·46
$\frac{sc\ +\ +}{+\ cv\ v}$	21	44	52·4044	0·21
$\frac{sc\ cv\ +}{+\ +\ v}$	20	42	53·8200	0·11
$\frac{sc\ +\ v}{+\ cv\ +}$	25	48	49·0052	0·44

Heterogeneity of the segregations of the replicates within each of the four maternal genotypes was tested (table 2) (Fisher, 1950).

As these χ^2 (table 2) values are not significant, the data can be regarded as homogeneous within each maternal genotype.

Each pair of complementary genotypes is represented once in the offspring of each triply heterozygous female parent. The two members

TABLE 3

Complementary pair	D.F.	Total χ^2	χ^2_1 on total	D.F.	Heterogeneity χ^2
E	4	27·9163 †	26·1523 †	3	1·7646
F	4	18·1636 †	13·4119 †	3	4·7517
G *	3	21·5455 †	17·7963 †	2	3·7519
H	4	11·7654 ‡	6·8667 †	3	4·8987

* In G the double recombinant class was added to one of the single recombinant classes as it was very small.

† Significant at P = 0·01.

‡ Significant at P = 0·02.

of the pair, assuming no viability or other disturbance, should be in a 1 : 1 ratio. Assuming a 1 : 1 ratio, a χ^2 test may be carried out for each pair of complementary genotypes for each of the four heterozygous female parents separately, and also on the grand totals of each complementary pair. The difference between the sum of the four χ^2 values for one degree of freedom each, and the χ^2_1 on the grand total will give an approximate heterogeneity χ^2 for each complementary pair (table 3).

From table 3 it can be concluded that members of each complementary pair are not in a 1 : 1 ratio and that deviations from the 1 : 1 ratio are homogeneous. This result suggests a consistent viability disturbance in the data.

From the marginal totals of the observed data (table 1) proportionate expectations were calculated for each cell of the 4×4 table (table 4).

TABLE 4

Expected frequencies

1598·3338	309·4207	479·6785	16·5670	2404·0000
1617·6148	313·1533	485·4650	16·7669	2433·0000
1376·2691	266·4313	413·0344	14·2653	2070·0000
1678·7823	324·9947	503·8221	17·4008	2525·0000
6271·0000	1214·0000	1882·0000	65·0000	9432·0000

Comparison of these expectations with the observed data (table 1) gives a general test of disturbance. In this case χ^2 was 54·8798 for nine degrees of freedom which is highly significant.

Viability differences between the pairs of genotypes E, F, G, H, which may have disturbed the linkage relationships will become evident by comparison of observed with the expected frequencies (Fisher, 1949), thus :

Complementary pair	Expected	Observed	χ^2
E	2341·9223	2409	1·9212
F	2445·1229	2389	1·2882
G	2197·7092	2248	1·1508
H	2447·2456	2386	1·5328
	9432·0000	9432	

giving $\chi^2 = 5{\cdot}8930$ for three degrees of freedom such that $0{\cdot}1 < P < 0{\cdot}2$. Using Fisher's (1949) method a more exact χ^2_3 was 12·6078 which is significant at $P = 0{\cdot}01$. This viability χ^2_3 is, however, a small portion of the total $\chi^2_9 = 54{\cdot}8798$.

4. REMOVAL OF VIABILITY DISTURBANCE—METHOD

As a first approximation, the method of Fisher (1949) is appropriate to remove viability disturbances, but the following iterative method due to Dr A. R. G. Owen provides a solution to any desired degree of accuracy.

Let p, q, r, s be the frequencies of the four modes of gamete formation and a, b, c, d be unknowns proportional to the number of zygotes found in each mating type (heterozygous female parent). Let u, v, w, t, be the average viability of the complementary pairs E. F, G, H.

The definitions can be standardised by :

$$\begin{aligned} u+v+w+t &= 4 \\ a+b+c+d &= 1 \\ p+q+r+s &= 1 \end{aligned}$$

Expectations can now be drawn up :

$$n\mathrm{K} \times \begin{cases} apu & aqv & arw & ast \\ bpv & bqu & brt & bsw \\ cpw & cqt & cru & csv \\ dpt & dqw & drv & dsu \end{cases}$$

such that n = number bred, and $\lambda = \frac{1}{\mathrm{K}} = apu + \ldots cqt \ldots + dsu$, 16 terms in all.

Let the observations divided by n be

a_1	a_2	a_3	a_4	A
b_1	b_2	b_3	b_4	B
c_1	c_2	c_3	c_4	C
d_1	d_2	d_3	d_4	D
P	Q	R	S	1

where row and column totals are as shown and U, V, W, T, are the totals of the pairs E, F, G, H.

The following transformations can be made :

$$\begin{aligned} x_1 &= a+b+c+d & 4a &= x_1+x_2+x_3+x_4 \\ x_2 &= a-b+c-d & 4b &= x_1-x_2+x_3-x_4 \\ x_3 &= a+b-c-d & 4c &= x_1+x_2-x_3-x_4 \\ x_4 &= a-b-c+d & 4d &= x_1-x_2-x_3+x_4 \end{aligned}$$

$$\begin{aligned} y_1 &= p+q+r+s & 4p &= y_1+y_2+y_3+y_4 \\ y_2 &= p-q+r-s & 4q &= y_1-y_2+y_3-y_4 \\ y_3 &= p+q-r-s & 4r &= y_1+y_2-y_3-y_4 \\ y_4 &= p-q-r+s & 4s &= y_1-y_2-y_3+y_4 \end{aligned}$$

$$\begin{aligned} 4z_1 &= u+v+w+t & u &= z_1+z_2+z_3+z_4 \\ 4z_2 &= u-v+w-t & v &= z_1-z_2+z_3-z_4 \\ 4z_3 &= u+v-w-t & w &= z_1+z_2-z_3-z_4 \\ 4z_4 &= u-v-w+t & t &= z_1-z_2-z_3+z_4 \end{aligned}$$

and let $\mathrm{X}_1 = \mathrm{A}+\mathrm{B}+\mathrm{C}+\mathrm{D}$ $\quad \mathrm{Y}_1 = \mathrm{P}+\mathrm{Q}+\mathrm{R}+\mathrm{S}$

$\mathrm{X}_2 = \mathrm{A}-\mathrm{B}+\mathrm{C}-\mathrm{D}$ etc. $\quad \mathrm{Y}_2 = \mathrm{P}-\mathrm{Q}+\mathrm{R}-\mathrm{S}$ etc.

$$\begin{aligned} 4\mathrm{Z}_1 &= \mathrm{U}+\mathrm{V}+\mathrm{W}+\mathrm{T} \\ 4\mathrm{Z}_2 &= \mathrm{U}-\mathrm{V}+\mathrm{W}-\mathrm{T} \text{ etc.} \end{aligned}$$

Putting $x_1 = \mathrm{X}_1 = y_1 = \mathrm{Y}_1 = z_1 = \mathrm{Z}_1 = 1$ will give a set of

equations which by iteration will give a maximum likelihood solution to any desired degree of accuracy.

$$\lambda = 1 + x_2y_2z_2 + x_3y_3z_3 + x_4y_4z_4$$
$$x_2 = \lambda X_2 - y_2z_2 - x_3y_4z_4 - x_4y_3z_3$$
$$x_3 = \lambda X_3 - y_3z_3 - x_2y_4z_4 - x_4y_2z_2$$
$$x_4 = \lambda X_4 - y_4z_4 - x_2y_3z_3 - x_3y_2z_2$$

$$y_2 = \lambda Y_2 - x_2z_2 - y_3x_4z_4 - y_4x_3z_3$$
$$y_3 = \lambda Y_3 - x_3z_3 - y_2x_4z_4 - y_4x_2z_2$$
$$y_4 = \lambda Y_4 - x_4z_4 - y_2x_3z_3 - y_3x_2z_2$$

$$z_2 = \lambda Z_2 - x_2y_2 - z_3x_4y_4 - z_4x_3y_3$$
$$z_3 = \lambda Z_3 - x_3y_3 - z_2x_4y_4 - z_4x_2y_2$$
$$z_4 = \lambda Z_4 - x_4y_4 - z_2x_3y_3 - z_3x_2y_2$$

From these, values for a, b, c, d and p, q, r, s and u, v, w, t can be obtained. The values p, q, r, s will give the frequencies of modes of gamete formation from which, by addition, the recombination values may be calculated.

5. REMOVAL OF VIABILITY DISTURBANCE FROM THE DATA UNDER DISCUSSION

The method outlined in the last section gave values for p, q, r, s :

$$p = 0{\cdot}665{,}685$$
$$q = 0{\cdot}127{,}645$$
$$r = 0{\cdot}199{,}835$$
$$s = 0{\cdot}006{,}835$$

From these, the corrected recombination values shown in table 5 were calculated. These are compared with the crude values calculated directly from the observed data.

TABLE 5

Crude and corrected recombination values

	sc-cv	*cv-v*	*sc-v*
Crude	13·5602	20·6425	32·8244
Corrected	13·4480	20·6670	32·7480

The corrected values are not much different from the crude values. Corrected values calculated using Fisher's (1949) method, which is a first approximation only, gave values intermediate between the crude and corrected values in table 5 thus :

sc—cv	*cv—v*	*sc—v*
13·4965	20·6583	32·7828

A reconstructed table of expectations using the iterative solutions for p, q, r, s ; a, b, c, d, and u, v, w, t may now be drawn up (table 6). The entries in this table are the expected frequencies after the elimination of viability.

A useful check is easily made by seeing if the rows, columns, and totals E, F, G, H are the same in table 6 as before iteration. This

TABLE 6

Expected frequencies (viability removed)

1625·9907	279·6249	483·4746	14·8838	2403·9740
1596·9582	341·4333	476·5399	18·1089	2433·0403
1393·9628	240·5798	422·4752	12·9596	2069·9974
1654·0591	352·3816	499·5176	19·0500	2525·0083
6270·9708	1214·0196	1882·0073	65·0023	9432·0000

check is a direct consequence of the maximum likelihood method used in the elimination of viability (Section 4).

Comparing the entries in table 6 with the observed values (table 1), gave a $\chi^2 = 41{\cdot}3924$ for six degrees of freedom. Three degrees of freedom were lost in the estimation and removal of viability interaction.

Thus we have :

DF	χ^2	
9	54·8798	Comparing expected and observed frequencies.
6	41·3924	Comparing expected and observed frequencies after removing viability.
3	13·4874	Viability.

TABLE 7

Maternal genotype	Recombination fractions		
	sc-cv	*cv-v*	*sc-v*
(i) *sc cv v*/+ + + .	10·6073±0·6199	20·4659±0·8229	30·0749±0·9353
(ii) *sc* + +/+ *cv v* .	12·2072±0·6637	20·5919±0·8199	31·8948±0·9449
(iii) *sc cv* +/+ + *v* .	14·1546±0·7662	21·3527±0·9007	34·0580±1·0416
(iv) *sc* + *v*/+ *cv* + .	17·1881±0·7508	20·2772±0·8001	35·3267±0·9512

In an attempt to detect factors responsible for this highly significant disturbance $\chi^2_6 = 41{\cdot}3924$ recombination values for each maternal genotype were calculated (table 7). The recombination values for the segment *sc—cv* were found to vary significantly according to the maternal genotype but in the segment *cv—v* there is no such variation. The variable recombination values for the entire segment *sc—v* reflect the variation in segment *sc—cv*. From examination of table 7 it will be evident that the data from each of the four maternal genotypes should be treated separately.

In this experiment environmental and genetic factors likely to cause variation were controlled. That such measures were effective can be seen from the homogeneity of families within each mating type (table 2). If a lethal or structural re-arrangement had affected part of the experiment it would be expected that the heterogeneity χ^2 values (table 3) for comparisons within each complementary pair would be significant and probably the values in table 2 also. However, if such a phenomenon had affected all the members of one female genotype equally the χ^2 values for table 2 would be expected to be non-significant. It can be concluded from the lack of heterogeneity in both of these tests that there was no lethal or structural re-arrangement affecting the data.

Dubinin (1933) discusses step-allelomorphism of the achaete-scute gene which would lead to misclassification if present. The scute gene that was used was not of variable manifestation and there was no indication of any error in breeding due to misclassification.

TABLE 8

Kosambi coefficients

Maternal genotype	K	χ^2_1 for deviation from unity
sc cv v/+ + + . .	0·3800±0·1011	4·5502
sc + +/+ *cv v* . .	0·2820±0·0854	5·6480
sc cv +/+ + *v* . .	0·3512±0·0903	6·4076
sc + *v*/+ *cv* + . .	0·4342±0·0833	8·7811
Whole experiment . .	0·3750±0·0464	25·7191

6. KOSAMBI COEFFICIENTS

Kosambi coefficients (Owen, 1953) may be calculated using the formula :

$$K = \frac{n^2 n_{12}}{2n_1 n_2 n_{1+2}}$$

where $n_1 = a_1 + a_{12}$
$n_2 = a_2 + a_{12}$
$n_{1+2} = a_1 + a_2$
$n_{12} = a_{12}$

and a_1 show recombination in segment 1 ($sc-cv$)
a_2 show recombination in segment 2 ($cv-v$)
a_{12} show simultaneous recombination in both segments.

To test departure of $\hat{K}$ from unity we may calculate

$$\chi^2_1 = \frac{(\hat{K}-1)^2}{V(K)}$$

where V(K) is the sampling variance at K = 1 (Owen, 1953). These tests are tabulated in table 8.

Despite the variation in the recombination fraction according to

maternal genotype, the four K values, which are all significantly less than unity (table 8), do not differ significantly from each other. Low K values such as these are expected for the distal end of the long arm of a chromosome on the basis of Owen's theory of genetic recombination (Owen, 1950).

7. DISCUSSION

A brief mention of the earlier use of orthogonal design in three-point data is appropriate. Bridges and Morgan (1916, 1919, 1923) published three-point data in *Drosophila* using this design. They assumed that tabulation of their data into a 4×4 Latin square would in itself remove spurious effects without further calculation. If only one gene is of lowered viability, calculation of recombination values from the column totals of the observed data is accurate, but if more than one gene is of lowered viability preliminary calculation of the type described by Fisher (1949) or the method outlined in this paper must be employed. There were no tests to detect differential viability or any other type of disturbance in these early data. It cannot be ascertained to what degree the environment was controlled in these experiments but there is frequently heterogeneity between individual matings and in some cases heterogeneity between members of a pair of complementary genotypes. In maize, Anderson (1921) published data arranged as a 4×4 Latin square, but they are heterogeneous probably due to misclassification which was mentioned as a source of error by the author.

More recently Wallace (1947, 1957) has published homogeneous data for the house mouse. In the former paper recombination values significantly greater than 50 per cent. were detected in the sex chromosome and the latter paper refers to an experiment using three markers of linkage group V to obtain accurate interference data.

It can be concluded that much of the early three-point linkage data is not of great accuracy, either because of heterogeneity, or because of some disturbance, undetected due to the lack of orthogonality. Wallace's recent work on mice is, of course, excluded from this criticism for it shows how these difficulties may be overcome.

We have at present little knowledge as to variation produced in the estimate of recombination value caused by extraneous genetic and environmental factors. In the experiment described in this paper, factors likely to cause heterogeneity were controlled as much as possible.

The orthogonal 4×4 Latin square design used in the analysis enabled viability disturbances to be removed by the method given in this paper, so as to obtain good estimates of the recombination values. This design is most efficient when the total progeny from each of the four mating types are approximately equal. In the data under discussion totals were 2404, 2433, 2070 and 2525 giving tolerable efficiency.

As well as a viability disturbance in the data, there was found to be a significant variation in recombination according to the arrangement of the genes in the maternal genotype. This variation occurs in the distal segment of the chromosome only, namely *sc—cv*, and not in the segment *cv—v*. The variation in this segment cannot be ascribed to the coupling and repulsion arrangement of the genes in the parent, since, as can be seen from table 8, maternal genotypes (i) and (iii) are in coupling for *sc—cv*, and (ii) and (iv) in repulsion, and the members of each pair differ greatly. The genes *cv—v* are in coupling in (i) and (ii) and in repulsion in (iii) and (iv) ; the members of each pair are unequal and are only sub-significantly different. Even so, the recombination value varies sufficiently to regard the offspring from the four different maternal genotypes as separate entities.

This phenomenon is in all probability due to a type of position effect dependant upon the arrangement of the genes in the heterozygote. Such a position effect may, of course, be restricted to the distal end of the chromosome. Equally careful tests in other regions of the same and different chromosomes should throw light on this interpretation.

8. SUMMARY

1. An orthogonal three-point linkage test was carried out in the sex chromosome of *Drosophila melanogaster*.

2. Significant differences in genotypic viabilities were found. Allowance was made for these differences in estimating recombination values for the three segments.

3. The estimates of the recombination values so obtained were found to vary according to the arrangement of the genes in the heterozygous parent. This may be interpreted as a position effect.

4. Kosambi values were calculated for each maternal genotype. These were found to be homogeneous and all significantly less than unity.

5. A brief mention is made of adequate control and orthogonal design in three-point linkage studies. Without these precautions the position effect discussed could not have been detected.

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THE THEORY OF GENETICAL RECOMBINATION: EFFECT OF CHANGING THE $\frac{1}{4}\chi^2_4$ INTERCEPT LENGTH DISTRIBUTION

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1. INTRODUCTION

The modern conception of the hereditary process, or the "chromosome theory of inheritance" as it is sometimes referred to, rests essentially on two broadly based facts. First, hereditary factors are readily classifiable into well-defined groups (known as linkage groups) according as they segregate independently or not; and secondly, the number of these groups in any species corresponds exactly to the haploid complement of chromosomes characteristic of the species in question. In terms of this conception the precise specification of the genetic constitution of an organism is achieved by assigning to each of its genes a certain location on a linkage map. The unit of measure in terms of which this might best be achieved is by no means obvious and it is only in the last few years in fact that a satisfactory metric has been put forward. Before describing this it is perhaps worthwhile recalling in very brief outline the attempts to develop a consistent theory of genetical recombination. Morgan propounded the cross-over theory in 1911. On the basis of this theory the segregation ratios of genetic factors belonging to the same linkage group were attributed to the exchange of chromosomal material during the meiotic process, and it was conjectured that the proportion of recombinants was in some way related to the distance apart of the genes on the chromosome. Subsequent research showed this to be an excellent first approximation where the recombination fractions were small, but that its effectiveness diminished as the recombination fraction increased. The recombination fraction could not therefore be regarded as a satisfactory metric and was eventually superseded by the map distance. Defined as the average number of cross-overs in an interval this was clearly additive, but it had the disadvantage of being unobservable, so that necessarily therefrom attention was focused on clarifying the precise relation between it and the observable recombination fractions. The difference between the map distance, x say, and the recombination fraction, y say, is due to two causes, multiple crossing-over and genetic interference, and it was the second of these which presented the main obstacle to progress. It was perhaps not very surprising therefore that the first relation to be established (Haldane, 1919) was based on the simplifying assumption of no interference; namely,

$$y(x) = \tfrac{1}{2}(1 - e^{-2x}).$$

The attempts to establish relations of wider applicability have been more numerous. Perhaps the best of them is Kosambi's empirical relation,

$$y(x) = \tfrac{1}{2} \tanh 2x.$$

In many organisms, particularly for describing gene loci on median segments in long arms, this is a very useful relation ; so much so that one feature to be expected of any theory of genetical recombination is that it should agree with Kosambi's relation over median ranges. Even so in other respects it has many serious shortcomings (see section 5) and this rather unsatisfactory state of affairs remained until the combined work of Fisher *et al.* (1947) and Owen (1949). Their theory is not dependent on any particular conception of the meiotic process ; it is simply formulated in terms of the probabilities of cross-overs occurring, and allows the observed recombination fractions themselves to provide the criterion as to whether a given probability distribution is well chosen or whether it should be altered so as to describe more accurately what has been observed.

2. THE GENERAL THEORY OF GENETICAL RECOMBINATION

This theory starts from the point of view that recombination between two loci takes place when, in terms of a single strand, an odd number of exchange points occurs between them during meiosis. The recombination fraction therefore is the probability that 1, or 3, or 5, or in general an odd number of interchanges has taken place, so that denoting the recombination fraction by y and using an obvious notation for the probabilities,

$$y = p_1 + p_3 + p_5 + \ldots = \sum_r p_{2r+1} \quad . \quad . \quad . \quad (1)$$

On the other hand the map distance, defined as the average number of exchange points,

$$x = 0p_0 + 1p_1 + 2p_2 + 3p_3 + \ldots = \sum_r rp_r \quad . \quad . \quad (2)$$

adequately supplies a consistent measure of location on the linkage map.

In a more general way, for the case of several loci on a finite arm, if we let $p_{r_1 r_2 \ldots r_n}$ denote the joint probability of just r_1, r_2, ..., r_n exchange points in each of say n genetically distinguishable segments then for the kth segment, the map distance,

$$x_k = \sum_{all r_k} r_k p_{r_1 \ldots r_k \ldots r_n}$$

= the expected number of exchange points in the kth segment, and the recombination fraction,

$$y_k = \sum_{odd r_k} p_{r_1 \ldots r_k \ldots r_n}$$

= the probability of an odd number of exchange points in the kth segment.

This simple and direct approach is the basis of the theory of genetical recombination developed in recent years by Fisher and Owen. It requires only the specification of the probability distribution of the exchange points on a single strand after meiosis, and is not dependent in any way on the particular manner in which a single exchange point occurs. Since the detail of this process is unobservable, this is an advantage as the theory would otherwise contain a formal aspect, *i.e.* one not testable by observation. Like many recent advances in biology during the past few decades it recognises variability and treats it as a whole, realising that what may happen in a particular instance depends on a large number of factors, which are either unknown or too complex to be treated individually even if they were known, while all that is necessary very often is the statistical regularity

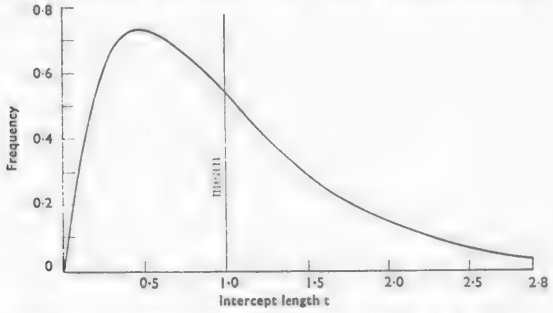


FIG. 1.—The intercept length distribution, $\frac{1}{2}\chi^2_4(t)dt = 4te^{-2t}dt$ having a variance $= \frac{1}{2}$.

which can be *observed* and *measured*. Evidently, therefore, the only essential requirement of this theory is a specification of the intercept length distribution between successive exchange points on a single strand ; from this the probabilities used in (1) and (2) can be inferred. In this feature lies the great flexibility of the theory ; one has only to find a distribution consistent with the observed recombination fractions and this will automatically subsume the composite effect of genetic interference. Furthermore the existence of a satisfactorily postulated form for the intercept distribution embodies the entire observational record of recombination fractions in an organised form and therefore provides an empirically established piece of knowledge for which future, more detailed investigation of genetic interference must account. Therefore, apart from allowing a consistent description of observed recombinations, which it does without prejudging the mechanism of genetic interference, it summarises in a compact way the observational experience with which any possible postulated mode of interference must accord.

To set up the requisite probabilities used in (1) and (2) Fisher recognised that there is nothing sacrosanct about the map distance.

(As Owen (1950) has demonstrated, there are an infinite number of possible additive metrics.) He chose, therefore, to define the intercept length distribution in terms of a metric in which it was supposed that the interference was uniform ; mathematically there is everything to be gained by adopting this procedure. If we denote this metric by t then the postulated form he chose,* is given by the $\frac{1}{4}\chi^2_4$ distribution,

$$\tfrac{1}{4}\chi^2_4(t)dt = 4te^{-2t}dt \quad . \quad . \quad . \quad . \quad (3)$$

This is shown graphically in fig. 1.

In terms of the auxiliary metric t one may express the probabilities p_r, and hence x and y, for

$$y = p_1(t) + p_3(t) + p_5(t) + \ldots = y(t)$$

and similarly $x = x(t)$.

Perhaps the first reaction of the reader is that the $\frac{1}{4}\chi^2_4(t)$ distribution is rather arbitrary, but it is the purpose of this paper to show in a summary fashion † that if only a small departure from the essential form of the $\frac{1}{4}\chi^2_4(t)$ distribution is made, then this leads to results which are directly at variance with current genetical experience, and that therefore the $\frac{1}{4}\chi^2_4(t)$ distribution must simulate the actual intercept distribution rather closely.

3. THE $\frac{1}{4}\chi^2_4(t)$ THEORY

In 1951 Fisher showed that the frequencies of all recombination classes among any number of marked loci on a finite arm can be expressed in terms of the following four functions,

$$\left.\begin{aligned} \alpha(t) &= 1 + \frac{t^4}{4!} + \frac{t^8}{8!} + \ldots . \\ \beta(t) &= t + \frac{t^5}{5!} + \frac{t^9}{9!} + \ldots . \\ \underline{\alpha}(t) &= \frac{t^2}{2!} + \frac{t^6}{6!} + \frac{t^{10}}{10!} + \ldots . \\ \underline{\beta}(t) &= \frac{t^3}{3!} + \frac{t^7}{7!} + \frac{t^{11}}{11!} + \ldots . \end{aligned}\right\} \quad . \quad . \quad (4)$$

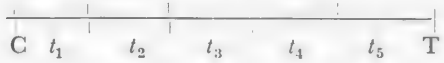
Note that in this notation t represents *twice* the metrical length. If products of these are formed in a certain combinatorial way (Fisher, 1951) then the frequency of each recombination class is readily deduced. However, for the purpose of this paper, it is more convenient to adopt a slightly different procedure. Suppose we have an arm of

* The $\frac{1}{4}\chi^2_4$ distribution was suggested by Owen (1949) as a mathematically more convenient mimic of the $\frac{\pi}{2}\tanh(\frac{1}{2}\pi s)\operatorname{sech}(\frac{1}{2}\pi s)ds$ distribution originally proposed by Fisher (1947).

† For the detailed analysis the reader is referred to Payne (1956).

five segments defined by four internal markers between the centromere and terminus ; in addition consider the 2×2 matrix.

$$\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix} \quad . \quad . \quad . \quad . \quad . \quad (5)$$



To form the frequency of non-recombinants in all five segments we write down the matrix product,

$$\overset{\leftarrow t_1 \rightarrow}{\begin{pmatrix} \alpha & \beta \\ 0 & 0 \end{pmatrix}} \overset{\leftarrow t_2 \rightarrow}{\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix}} \overset{\leftarrow t_3 \rightarrow}{\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix}} \overset{\leftarrow t_4 \rightarrow}{\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix}} \overset{\leftarrow t_5 \rightarrow}{\begin{pmatrix} \alpha & 0 \\ \underline{\beta} & 0 \end{pmatrix}}$$

that is, we associate the matrix $\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix}$ with each *internal* segment, its first *row* with the segment proximal to the centromere, and its first *column* with the terminal segment. If recombination occurs in any segment, *e.g.* in the second, the appropriate frequency for that particular class is given by exchanging $\begin{pmatrix} \underline{\alpha} & \underline{\beta} \\ \beta & \underline{\alpha} \end{pmatrix}$ for $\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix}$ in the second segment (*i.e.* we merely interchange the elements of the matrix according to whether they are underlined or not). Thus we may regard,

$\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix}$ as the " non-recombination matrix "

and $\begin{pmatrix} \underline{\alpha} & \underline{\beta} \\ \beta & \underline{\alpha} \end{pmatrix}$ as the " recombination matrix ".

According as we use one or the other in each segment we thus obtain in a straightforward way the full set of 2^5 frequencies ($2^5 - 1$ recombinant, 1 non-recombinant) appropriate to there being recombination or non-recombination in each of the five segments. Incidentally we note that the sum of recombinants and non-recombinants in a given segment is found by adding,

$$\overset{\leftarrow t \rightarrow}{\begin{pmatrix} \alpha & \beta \\ \underline{\beta} & \alpha \end{pmatrix}} + \overset{\leftarrow t \rightarrow}{\begin{pmatrix} \underline{\alpha} & \underline{\beta} \\ \beta & \underline{\alpha} \end{pmatrix}} = \overset{\leftarrow\!\!- t -\!\!\rightarrow}{\begin{pmatrix} \alpha + \underline{\alpha} & \beta + \underline{\beta} \\ \underline{\beta} + \beta & \alpha + \underline{\alpha} \end{pmatrix}} = \begin{pmatrix} \cosh t & \sinh t \\ \sinh t & \cosh t \end{pmatrix}$$

so that the sum of all recombination frequencies is just,

$$\overset{\leftarrow\!\!- t_1 -\!\!\rightarrow}{\begin{pmatrix} \cosh & \sinh \\ 0 & 0 \end{pmatrix}} \overset{\leftarrow\!\!- t_2 -\!\!\rightarrow}{\begin{pmatrix} \cosh & \sinh \\ \sinh & \cosh \end{pmatrix}} \ldots\ldots\ldots \overset{\leftarrow t_5 \rightarrow}{\begin{pmatrix} \cosh & 0 \\ \sinh & 0 \end{pmatrix}} = \cosh(t_1 + t_2 + \ldots + t_5)$$

or simply cosh T, where T is twice the total metrical length of the arm. This will therefore be a constant divisor for finding absolute frequencies.

In the same way that the expressions are built up for the recombination frequencies from the " recombination " and " non-recombination " matrices, so we can build up the expression for the map distance from the following " map distance matrix."

$$\tfrac{1}{2}\begin{pmatrix} t\sinh t & t\cosh t-\sinh t \\ t\cosh t+\sinh t & t\sinh t \end{pmatrix}$$

4. THE $\frac{1}{6}\chi^2_6$ THEORY

The above summary of a method for quickly finding $y(t)$ and $x(t)$ in the $\frac{1}{4}\chi^2_4$ case can readily be extended to other metrics. Since we are anxious to determine the genetic consequences implicit upon a slight change in the $\frac{1}{4}\chi^2_4$ distribution of intercept length we now investigate the case of the $\frac{1}{6}\chi^2_6$ distribution. Explicitly,

$$\tfrac{1}{6}\chi^2_6(t)dt = \frac{27}{2}\,t^2e^{-3t}dt.$$

This is compared graphically with the $\frac{1}{4}\chi^2_4$ distribution in fig. 2.

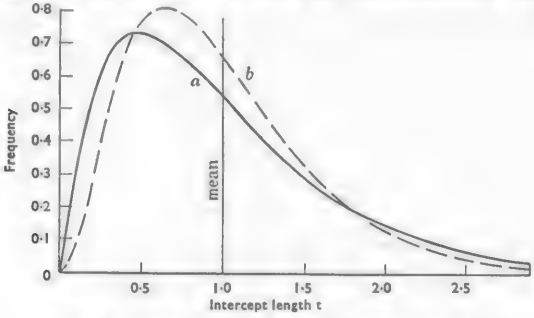


FIG. 2.—Curve (*a*) $\frac{1}{4}\chi^2_4(t) = 4te^{-2t}$ (variance = $\frac{1}{2}$).
Curve (*b*) $\frac{1}{6}\chi^2_6(t) = \frac{27}{2}t^2e^{-3t}$ (variance = $\frac{1}{3}$).

The expressions for $x(t)$ and $y(t)$ in this case can be formulated in terms of the following six functions,

$$\left.\begin{aligned}
\alpha(t) &= 1 + \frac{t^6}{6!} + \frac{t^{12}}{12!} + \cdots \\
\beta(t) &= \frac{t}{1!} + \frac{t^7}{7!} + \frac{t^{13}}{13!} + \cdots \\
\gamma(t) &= \frac{t^2}{2!} + \frac{t^8}{8!} + \frac{t^{14}}{14!} + \cdots \\
\underline{\alpha}(t) &= \frac{t^3}{3!} + \frac{t^9}{9!} + \frac{t^{15}}{15!} + \cdots \\
\underline{\beta}(t) &= \frac{t^4}{4!} + \frac{t^{10}}{10!} + \frac{t^{16}}{11!} + \cdots \\
\underline{\gamma}(t) &= \frac{t^5}{5!} + \frac{t^{11}}{11!} + \frac{t^{17}}{17!} + \cdots
\end{aligned}\right\} \quad . \quad . \quad (6)$$

Note that in this notation t represents *thrice* the metrical length. The expressions for $x(t)$ and $y(t)$ can now be formed in exactly the same way as for the $\frac{1}{4}\chi^2_4$ case, but using instead the following matrices,

$$\begin{pmatrix} \alpha & \beta & \gamma \\ \underline{\gamma} & \alpha & \beta \\ \underline{\beta} & \underline{\gamma} & \alpha \end{pmatrix} \text{ is the " non-recombination matrix "}$$

$$\begin{pmatrix} \underline{\alpha} & \underline{\beta} & \underline{\gamma} \\ \gamma & \underline{\alpha} & \beta \\ \beta & \gamma & \underline{\alpha} \end{pmatrix} \text{ is the " recombination matrix "}$$

and $\frac{1}{3}\begin{pmatrix} t\phi & t\delta-\epsilon & t\epsilon-2\phi \\ t\epsilon+\phi & t\phi & t\delta-\epsilon \\ t\delta+2\epsilon & t\epsilon+\phi & t\phi \end{pmatrix}$ is the " map distance matrix ",

where, $\delta = \alpha+\underline{\alpha}$, $\epsilon = \beta+\underline{\beta}$ and $\phi = \gamma+\underline{\gamma}$.

By associating one or other of the first two matrices with each internal segment and the first row and column with the segments proximal to the centromere and terminus respectively, the full set of recombination frequencies can be obtained. Similarly the map distances are obtainable from the third matrix.

We note that each matrix is of order 3×3; for this reason no confusion is likely to arise from the notation adopted for the sets of functions (4) and (6), and something is perhaps gained by observing the natural extension of the way in which the matrices can be built up. Finally we note that in this case the sum of all possible frequency classes is just $\delta(\tau)$; this is therefore the constant divisor required to obtain absolute frequencies.

5. COMPARISON OF THE TWO DISTRIBUTIONS

With the above summary of how, within the framework of the general theory of genetical recombination, the required relation between x and y can be found, ($x = x(t)$, $y = y(t)$ expresses it parametric form), in the cases of the two assumed forms for the intercept distribution (which we note subsumes the effects of multiple crossing-over and genetic interference) we can now pass on to compare the implicit genetic consequences.

To do this we adopt a suitable convention for representing the level of interference induced over a finite arm already used by Fisher.* Taking an arm length of 90 cM. between centromere and terminus we mark off segments at distances such that the recombination fraction for each segment is 10 per cent. The Kosambi coefficient † is then

* A chart, based on an arm length of 60 cM. is exhibited as a wall diagram in the Dept. of Genetics, Cambridge.

† The Kosambi coefficient of interference is given by

$$K_{12} = \frac{y_1 - y_2 - y_{1+2}}{4y_1y_2y_{1+2}} = \frac{C}{2y_{1+2}}$$

where C is the coefficient of coincidence between segments 1 and 2.

calculated for each adjacent pair of segments and plotted against the central marker as abscissæ. The matrix method of the previous sections facilitates the calculation of the necessary recombination fractions and map distances. To illustrate the method consider the calculation of a single point on the graph. Let two segments, 1 and 2 say, be specified by $t_1 = 27$, $t_2 = 40$ and $t_3 = 51$ cM. (these points refer to the standardised metric ; *i.e.* put $2t$ for t in the formulæ of the previous sections).

For the $\frac{1}{2}\chi^2_4$ case the appropriate expression for the recombination fraction $y(t_1t_2)$ is as follows.

$$y(t_1t_2) = (\cosh \sinh)\overset{\leftarrow 2t \rightarrow}{\begin{pmatrix} \alpha & \beta \\ \beta & \alpha \end{pmatrix}}\overset{\leftarrow 2(t_2-t_1) \rightarrow}{\begin{pmatrix} \cosh \\ \sinh \end{pmatrix}}\Big/ \cosh 2T \overset{\leftarrow 2(T-t_3) \rightarrow}{=} \frac{A.B.C.}{\cosh 2T}, \text{ say.}$$

Hence if y_1 and y_2 refer to the respective recombination fractions of segments 1 and 2 then we have (simply substituting in the above for the appropriate t_1 and t_2) that,

$$y_1(27, 40) = \overset{\leftarrow A \rightarrow}{\begin{Bmatrix} 1{\cdot}1494 \\ 0{\cdot}5666 \end{Bmatrix}}\overset{\leftarrow B \rightarrow}{\begin{Bmatrix} 0{\cdot}1861178 \\ 0{\cdot}0225182 \end{Bmatrix}}\overset{\leftarrow C \rightarrow}{\begin{Bmatrix} 1{\cdot}6685 \\ 1{\cdot}3356 \end{Bmatrix}} = 0{\cdot}0997$$

$$y_2(40, 51) = \begin{Bmatrix} 1{\cdot}3374 \\ 0{\cdot}8881 \end{Bmatrix}\begin{Bmatrix} 0{\cdot}2277512 \\ 0{\cdot}0238657 \end{Bmatrix}\begin{Bmatrix} 1{\cdot}4128 \\ 0{\cdot}9981 \end{Bmatrix} = 0{\cdot}1011$$

These are as near to 10 per cent. as the tables* permit.

whilst $$y_{1+2}(27, 51) = \begin{Bmatrix} 1{\cdot}1494 \\ 0{\cdot}5666 \end{Bmatrix}\begin{Bmatrix} 0{\cdot}4045187 \\ 0{\cdot}0864691 \end{Bmatrix}\begin{Bmatrix} 1{\cdot}4128 \\ 0{\cdot}9981 \end{Bmatrix} = 0{\cdot}1925.$$

Thus the Kosambi coefficient K_{12} is,

$$K_{12} = \frac{y_1+y_2-y_{1+2}}{4y_1y_2y_{1+2}} = 1{\cdot}070.$$

This is therefore plotted against the central marker $t_2 = 40$ cM as abscissa ; but this point corresponds to a map distance of,

$$x(t) = \tfrac{1}{2}\begin{pmatrix} 2t \sinh 2t & 2t \cosh 2t - \sinh 2t \\ 0 & 0 \end{pmatrix}\begin{pmatrix} \cosh 2(T-t) & 0 \\ \sinh 2(T-t) & 0 \end{pmatrix}\Big/ \cosh 2T$$

$$= (2t \sinh 2t - \sinh 2t \sinh 2(T-t))\,/2 \cosh 2T$$

which for $t = 0{\cdot}40$ gives,

$$x(0{\cdot}40) = \{0{\cdot}8 \sinh (1{\cdot}9) - \sinh (0{\cdot}8) \sinh (1{\cdot}1)\}\,/3{\cdot}4177$$

$$= 20{\cdot}90 \text{ cM.}$$

$K = 1{\cdot}07$ is therefore plotted against $x = 20{\cdot}9$ cM as abscissa. The

* The segmental functions (4) are tabulated in Fisher and Yates' *Statistical Tables* (1953).

entire set of results obtained in the above manner are given in table I. The ordinate value $K_0 = 2{\cdot}78$ for $x = 0$ is derived on the assumption

TABLE 1

t . .	00·00	27·00	40·00	51·00	61·00	70·00	78·00	85·00	91·00
$x(t)$.	00·00	10·73	20·90	31·15	41·74	52·42	63·06	73·49	83·50
K . .	2·78	1·61	1·07	0·85	0·69	0·57	0·47	0·38	...

that there is no interference across the centromere so that the corresponding y_{1+2} refers to segments on either side of the centromere, and is therefore given by,

$$\begin{aligned} y_{1-2} &= y_1 + y_2 - 2y_1y_2 \\ &= 0{\cdot}1 + 0{\cdot}1 - 2(0{\cdot}1 \times 0{\cdot}1) \\ &= 0{\cdot}18, \end{aligned}$$

whence $K_0 = 2{\cdot}78$.

TABLE 2

t . .	00·00	36·67	49·33	58·67	66·00	72·33	77·67	82·00	86·00
$x(t)$.	00·00	10·27	20·29	30·18	39·88	50·04	60·21	69·87	80·05
K . .	2·78	0·88	0·34	0·18	0·11	0·07	0·04	0·03	...

A similar calculation applies to the $\frac{1}{6}\chi^2_6$ case ; the only difference is that $x(t)$ and $y(t)$ are built up from the 3×3 matrices instead of the

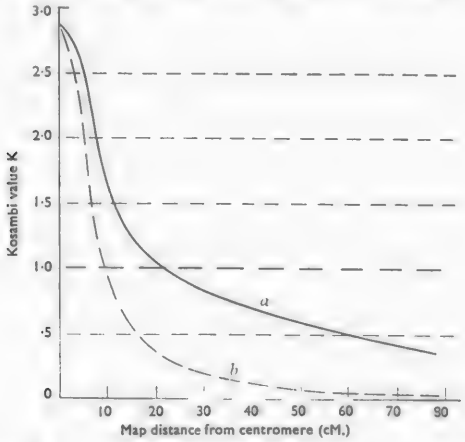


FIG. 3.—Values of K for pairs of segments, each with 10 per cent. recombination, plotted against the position of the central marker. Curve (*a*) corresponds to the $\frac{1}{4}\chi^2_4$ intercept distribution and curve (*b*) to the $\frac{1}{6}\chi^2_6$ distribution.

2×2. (Tables for the six functions (6) have been calculated by the author.) The results for the $\frac{1}{6}\chi^2_6$ case are summarised in table 2.

A graphical illustration of tables 1 and 2 is given in fig. 3.

One notices that the range of values predicted by the $\frac{1}{4}\chi^2_4$ theory is consistent with current genetical evidence, whilst those predicted by the $\frac{1}{6}\chi^2_6$ theory are of a much lower order. The fact that only a relatively slight departure from the $\frac{1}{4}\chi^2_4$ distribution (see fig. 2) is sufficient to imply very different K values from those which have been observed suggests that the $\frac{1}{4}\chi^2_4$ distribution simulates the actual intercept distribution rather closely.

6. RELATION TO KOSAMBI'S THEORY

Kosambi's empirically derived relation between the map distance and the recombination fraction is of the form,

$$y(x) = \tfrac{1}{2}\tanh 2x$$

from which it may be verified that the Kosambi coefficient is unity throughout the arm. This follows from the implicit addition formula,

$$y_{1+2} = \frac{y_1+y_2}{1+4y_1y_2}.$$

It also follows from this that for n loci only the recombination fractions between $\frac{1}{2}n(n-1)$ pairs of points can be derived, whilst there actually exist 2^n-1 recombination classes. Therefore this relation does not provide an adequate basis from which to infer all the gametic frequencies in the cases of four or more loci. Further, it requires that y be a monotonic function of x, sets an upper limit of 50 per cent. to the recombination fraction for any segment and implies uniform properties for a chromosome arm throughout its length. However, it is known to give good agreement in many cases for segments distal from the centromere on long arms, and it will be expected therefore of any theory (such as that based on the $\frac{1}{4}\chi^2_4$ or $\frac{1}{6}\chi^2_6$ distribution) that apart from overcoming the weaknesses of the Kosambi relation, it will agree closely with it over median ranges (where K is approximately unity). One may observe in fig. 3 that there is a range of values of x over which, for the $\frac{1}{4}\chi^2_4$ curve, K is near unity, *i.e.* the value given by Kosambi's relation. For this range one may therefore expect there to be good agreement between these theories, and correspondingly less good agreement between Kosambi's relation and the $\frac{1}{6}\chi^2_6$ theory. The relevant approximations are * given by the following expressions.

$$\tfrac{1}{4}\chi^2_4 : y(x) = \tfrac{1}{2}(1-e^{-2x}\cos 2x)$$

$$\tfrac{1}{6}\chi^2_6 : y(x) = \tfrac{1}{3}e^{-3x}.\{3\underline{\alpha}(3x)+2\underline{\beta}(3x)+2\underline{\gamma}(3x)+2\gamma(3x)+\beta(3x)\}$$

These are compared with Kosambi's relation in table 3. Both approximations agree fairly well with Kosambi over this range, though as we anticipated from fig. 3 the agreement is rather better in the $\frac{1}{4}\chi^2_4$ case.

In other respects the $\frac{1}{6}\chi^2_6$ analysis enjoys qualitative advantages similar to the $\frac{1}{4}\chi^2_4$ theory, in that it does not confine all recombination

* See Owen (1951), Payne (1956).

fractions below 50 per cent., nor ascribe uniform properties throughout the length of a finite arm, but does allow us to calculate any required recombination class frequency and provide a consistent measure of location in an additive metric. It will be interesting to see if there are any species where the theory of recombination based on the $\frac{1}{6}\chi^2_6$ distribution of intercept length, corresponding to more severe genetic interference, gives better agreement than that based on the $\frac{1}{4}\chi^2_4$ distribution. The simple experimental criterion suggested by fig. 3 should assist such investigation.

TABLE 3

x (cM)	Kosambi	$\frac{1}{4}\chi^2_4$	$\frac{1}{6}\chi^2_6$
0	0·000	0·000	0·000
4	0·040	0·040	0·040
8	0·079	0·079	0·080
12	0·118	0·118	0·120
16	0·155	0·155	0·159
20	0·190	0·191	0·198
24	0·223	0·226	0·235
28	0·254	0·258	0·272
32	0·283	0·289	0·307
36	0·308	0·317	0·340

7. SUMMARY

A simple algebraic method is given for obtaining the relation between the map distance x and the recombination fraction y in the cases of two assumed distributions for the intercept length between successive exchange points on a single strand (the $\frac{1}{4}\chi^2_4$ and $\frac{1}{6}\chi^2_6$ distributions). Since these correspond to different levels of genetic interference the interference levels are calculated in either case. It is clear from this that whereas the $\frac{1}{4}\chi^2_4$ distribution yields results in good agreement with current genetical evidence, the $\frac{1}{6}\chi^2_6$ distribution does not, and as the difference between these distributions is slight one is led to infer that the $\frac{1}{4}\chi^2_4$ distribution (due to Fisher and Owen) simulates the actual intercept length distribution rather closely.

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CHIMERAS AND MERISTEMS

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I. SHOOT APICAL MERISTEMS

In the higher plants most of the longitudinal growth and often much of the latitudinal growth takes place at the apices of shoots and roots. Because of this and the fact that plant embryos are difficult subjects for experiments, the arrangement and behaviour of the dividing cells of the apex have interested botanists for the last hundred years. The cells cannot be seen dividing so their planes and frequencies of division have to be inferred from such observations as the arrangement of cells, the thickness of cell walls, and by less direct means. Some of these last depend upon the interpretation of chimeras, which may be variegated plants, or graft hybrids, or have cells with different chromosome complements, and recently work on the apices of chimeras has produced several useful results.

Chromosome chimeras have been noted in roots (Navaschin, 1927 ; Kachidze, 1932 ; Juhl, 1953), but their stability is uncertain and it is in shoots, where the cell arrangement is more difficult to interpret, that the work has been useful.

In median sections of the apices of most plants there are layers of cells parallel to the surface. There are two theories about these layers. Hanstein's *histogen* theory assumed that the layers were tissue-specific, and separated them according to whether they gave rise to the epidermis, or cortex, or stele. This theory is not useful except for roots and some anomalous shoots because the pattern of layers is upset in the formation of leaf primordia and their vascular connexions. Modern views on shoot apices are dominated by a modified form of the *tunica-corpus* theory originally put forward by Schmidt (1924). This divides the meristem above the leaf primordia into an outer tunica of one or more layers where cell divisions are anticlinal, perpendicular to the surface, and an inner corpus where the dividing walls may be either anticlinal or periclinal or in any other plane. The corpus and each of the tunica layers, being independent of each other, behave as germ layers in the sense used by embryologists, but they are not histogens, that is, they are not tissue-specific.

Early workers thought that the number of tunica layers was constant, but most now recognise that it may vary with the age of the plant and the phase of the plastochron, the period of time between the formation of one leaf and the next. The theory depends upon the fact that a solid body, growing throughout, must increase its surface greatly in order to accommodate its increasing volume. The outer cells of a shoot apex accommodate this surface expansion by

anticlinal divisions. It is therefore to be expected that there will be a gradual increase in the predominance of anticlinal divisions away from the centre. Similarly the wider the apex becomes in each plastochron and in the life of each shoot the greater will be the number of layers dividing only anticlinally.

For these reasons it is often impossible by inspecting sections to say certainly how many tunica layers there are in a meristem. Nevertheless the tunica-corpus theory is a useful, simplified way of describing the behaviour of most Angiosperm shoot apices. There are, however, certain seed plants which do not have layered apices and to them it cannot be applied. In these the outer cells divide anticlinally and, in addition, periclinally and thus produce anticlinal rows of cells. The apex then appears like a fountain as in some cycads, or is irregular where periclinal divisions are rarer as in some conifers and Monocotyledons (Thielke, 1954).

At best the tunica-corpus concept describes only one aspect of the behaviour of cells in an apex above the youngest leaf primordia. Superimposed on the layering there are patterns of rates of mitosis and differentiation which give rise to various zonations. Where the tunica consists of more than one layer, periclinal divisions usually do occur in its inner layers in the formation of leaf primordia. The number of cell layers which go to form the leaves varies with the species.

2. CHIMERAS

Chimeras can arise spontaneously or can be induced by mitotic poisons or by grafting. Many cultivated plants once thought to be polyploid are now known to be chimeras (Blaser and Einset, 1948 ; Einset and Lamb, 1951 ; Dermen, 1951*a*). Those used in developmental anatomy are either spontaneously variegated or spontaneous or induced partial polyploids. Green plastids can mutate under direct or indirect nuclear control and produce colourless or pale plastids (Darlington and Mather, 1950). Plastids divide and grow and are passed to each cell generation with the cytoplasm. They are not necessarily divided equally between sister cells so that a cell with both green and colourless plastids can give rise to tissues whose cells contain only green or only colourless plastids. Variegated chimeras arise in this way. Some tissues do not develop chlorophyll even if the cells inherit potentially green plastids and variegation may result from causes other than inheritance of different kinds of plastids. In nature variegated chimerical seedlings do not usually survive, but in cultivation they and chimerical branches are often preserved and propagated.

Polyploid chimeras have to be examined microscopically for chromosome number, cell size, or nucleolus number. They are better than variegated chimeras for interpreting apices since the chimerical structure can be seen in sections of the apex as well as in the leaves.

In *Panicum* and other plants which have leaves produced from the outer layer of the tunica only (Thielke, 1951), stable, variegated periclinal chimeras could not be detected if they exist.

3. CHIMERICAL MERISTEMS

The polyploid chimeras produced by treatment of buds or seeds with colchicine are periclinally arranged and sometimes sectored as well. Their stability appears to vary with the species. Satina, Blakeslee and Avery (1940) produced periclinal chimeras in *Datura* shoots which appeared perfectly stable. Their plants had two tunica layers and they found three germ layers which were diploid, tetraploid or octoploid in various arrangements. One germ layer is the outer tunica layer, the second is the inner tunica layer, and the third is the corpus, and these remained completely independent. Baker (1943) obtained similar results in *Solanum* which has a single tunica layer and therefore two germ layers. Dermen (1947 and 1953*a*) found that in *Oxycoccus* and *Prunus persica* the germ layers were not so stable. In both plants there were normally three germ layers and the innermost could have both diploid and tetraploid cells presumably because the middle layer divided periclinally occasionally. In *Prunus* particular tissues could vary in their derivation.

Variegation can be maintained by both periclinal and sectorial forms of chimera. The behaviour of the apex of such plants has to be interpreted from the colour pattern in the leaves because plastids cannot be distinguished in the apices. Plants with leaves whose margins are of different colour from the centres are nearly always periclinal chimeras. Renner and Voss (1942) showed that *Prunus pissardii* var. *Hessei* was like this. The white margin is due to the periclinal division at the margin of the epidermis which is without green plastids. *Pelargonium* Freak of Nature is similar, but with a green margin round a white centre.

Thielke (1954) has recently obtained valuable results from white-over-green variegated chimeras in the Commelinaceæ. In *Commelina benghalensis* var. *foliis variegatis*, a plant having pale green leaves with white margins and also white stripes running into the tip, she showed that the outer layer of the tunica produced the leaf epidermis, the inner tunica layer produced the subepidermal layer of the mesophyll, and the corpus produced the central mesophyll in the green areas. The plant is a white-over-green periclinal chimera. The pale green parts of the leaf have chlorophyll in the central mesophyll and practically none in the outer mesophyll and epidermis. The white parts are due to a normal and an abnormal cause. At the white leaf margin the absence of underlying green tissues is caused by the normal absence of corpus derivatives, as in *Prunus pissardii*. The white stripes are due to the abnormal absence of corpus derivatives and the abnormal multiplication of the inner tunica layer, in other words, to a breakdown of the regular layering.

Tradescantia fluminensis var. *albostriata* is also a periclinal chimera and it is of similar appearance. Nevertheless, its apex behaves differently from that of *Commelina*. There is no tunica in some shoots, for some of the outer cells divide periclinally. The leaf primordia are formed sometimes from cells wholly derived from the outer layer and sometimes from inner tissue as well. The white stripes in the leaves are derived wholly from the outer layers and the green stripes are derived from inner tissue with one or two layers of the outer tissue. The leaf primordia in *Tradescantia* and many other Monocotyledons are very broad and allow sectors of one leaf to be constructed in different ways. The plant then is a periclinal chimera which is secondarily sectored.

Tradescantia albiflora var. *albovittata* is a periclinal chimera which is sectored primarily, *i.e.* mericlinal (Thielke, 1954). There is a single tunica layer and the cells of the corpus are arranged in regular horizontal rows between the distichous primordia. These rows remain discrete even in the leaf primordia and allow the leaf to have longitudinal stripes, each stripe derived from a cell which is genetically different from cells of the adjacent stripes. The single-layered tunica forms the epidermis only, and as this has no chlorophyll except in the guard cells, it does not obscure the white stripes. Axillary shoots from variegated plants of this species can be wholly white or wholly green because the angle subtended by the bud is smaller than that subtended by the axillant leaf.

Zebrina pendula var. *quadricolor* is similar except that the stripes are broader because the corpus cells are larger in relation to the width of the apex than in *Tradescantia albiflora*. Thielke (1955) has also described a variegated chimera of *Hemerocallis* where the origin of the sectoring in the discrete rows of cells of the corpus is particularly clear.

4. SIGNIFICANCE OF CHIMERAS FOR NORMAL ONTOGENY

The use of chimeras to investigate the behaviour of meristems must be subject to two important corrections.

(1) The chimerical form may behave differently from the normal form. There is plenty of evidence for this. The crumpled appearance of the leaves of some chimeras is presumably due to poor co-ordination in growth rates of the various layers. In the polyploid chimeras the larger polyploid or aneuploid cells may divide at different rates from diploid cells. Also the different kinds of cells need not compete in the same way as cells of a genetically homogeneous tissue. Dermen (1953*a*) in fact showed that the stability of the chimera depends upon how the diploid and tetraploid cells were distributed among the three germ layers in *Prunus persica*. In his material most of the chimerical types were not permanent, but those with the $2n-4n-4n$ and $4n-2n-2n$ arrangement were usually stable. It is especially important

to look for anomalous behaviour of meristems in studies on the homology of organs (Satina and Blakeslee, 1941, 1943; Dermen, 1953*b*).

In the variegated chimeras, too, there is evidence that cells that produce white or yellow tissues differ from those that produce green tissues not only in the primary chlorophyll difference, but also in secondary respects. In *Commelina benghalensis* the inner layer of the tunica of variegated plants consists of cells which are more elongated tangentially than those of the green plants. In this species, and in other members of the Commelinaceæ described by Thielke (1954), the leaf palisade cells were of different shapes in the green and yellow parts of the leaf. In *Prunus pissardii* the leaf epidermis divides periclinally and anticlinally in the variegated chimera, but only anticlinally in the normal form (Renner and Voss, 1942). In *Pelargonium* Freak of Nature the epidermis divides periclinally and anticlinally when there is yellow tissue underneath, but only anticlinally in green leaves, or in leaves where there is only a small central yellow patch, or in shoots which have lost the potentially green outer layer (Thielke, 1948). In *Sanseviera* the leaf margin can produce roots when it is green, but not when it is colourless except after applying indole acetic acid (Thielke, 1948). A more striking difference between a normal and a chimerical plant occurs in *Tradescantia fluminensis* var. *albostriata*. Variegated plants usually, but not invariably, have the anomalous apical structure described above. Wholly green shoots usually, but not invariably, have a normal one-layered tunica.

(2) Variegation in certain exceptional chimeras may be due not solely to the chimerical structure, but partly to physiological gradients. The possibility that this might be so can be seen in Thielke's paper on *Hemerocallis*. Her plants were mericlinal chimeras, and the single tunica layer, dividing anticlinally only, should not have shown the sectoring. Yet she found that the guard cells were not clearly green when over yellow mesophyll, but were green over green mesophyll. A similar bleaching effect on green tissue has been reported for the yellow tissue of some *Pelargonium* chimeras (Chittenden, 1927). The green flecks sometimes found in white tissue of *Pelargonium* Freak of Nature could be due to physiological causes though they are usually interpreted as due, either to islands of meristematic activity in the epidermis, or to their being mericlinal chimeras with a few green cells scattered in a white apex (Jones, 1934).

However, much useful information about the behaviour of apices can be found by studying chimeras. The fact that stable periclinal chimeras exist at all shows that the tunica layers are independent of each other and of the corpus. An understanding of the mechanical basis of the tunica-corpus theory leads us to expect variation in the stability of periclinal chimeras, and this is what is found. Some plants like *Datura* are perfectly stable; others are only partially so, and instability is always strongest in the inner layers. It was once

thought that the plants without a tunica could not produce stable periclinal chimeras and this accounted for their rarity in conifers, but Thielke has shown that periclinal divisions in the outer layer of the apex do not prevent a periclinal arrangement from becoming stable so long as there is a genetic discontinuity between two other layers of the apex.

This view has been criticised by Hejnowicz (1956) who wrongly supposes that periclinal divisions in the outer layer must eventually contribute to the corpus and so cause the shoot to become non-chimerical. Hejnowicz has also reported the existence of unstable periclinal chimeras in a conifer, *Juniperus sabina*. Its apparently green shoots have a white surface layer, and the chimera is unstable because the white surface cells divide periclinally at the apex and so invade the green inner tissue producing, first, a mericlinal chimera with white sectors and, later, a wholly white shoot. The plant does not lose its chimerical character because many new chimerical branches arise from the periclinal or mericlinal parts of shoots.

Another point of interest arises from the colchicine-induced polyploid chimeras. Their polyploid cells must always be derived from the frustration of anaphase in a cell which is undergoing mitosis. The polyploid germ layers are most likely derived from single mutant cells. These divide and their progeny take over the whole of the germ layer. This is also what happens when a non-chimerical shoot is produced from an unstable periclinal chimera (Dermen, 1951*b*). According to a recent French theory on the behaviour of apices the apical cells of the tunica do not divide and do not give rise to any new tissue at all in vegetative apices (Buvat, 1952, 1953 ; Lance, 1952, 1953). This cannot be true of periclinal chimeras such as those of *Datura*. The mutating cells which produce the polyploid germ layers must have been dividing during the colchicine treatment, and even if they were on the flanks of the meristem their progeny must divide at the apex in colonising the whole of the germ layer.

Another contribution to our knowledge of apices comes from Thielke's demonstration that the arrangement of cells as seen in transverse sections does reflect accurately the planes of their division. She showed (Thielke, 1954) that in the Commelinaceæ only those species with horizontal rows of cells in the apices between the distichous leaf bases produced mericlinal chimeras. It is interesting that among closely related plants there should be such diversity in the behaviour of the apical meristem. Mericlinal chimeras are often not stable in plants with a well developed tunica (Dermen, 1945). Meristematic activity tends to convert them to periclinal chimeras or to non-chimeras as might be predicted on the tunica-corpus theory. But in the Monocotyledons studied by Thielke the peculiar behaviour of the meristem associated with the distichous leaves subtending wide angles at the shoot axis preserves the stability of the sectorial pattern even when there is a tunica.

5. SUMMARY

1. Work on chimeras has been used in the study of the behaviour of shoot apical meristems.

2. The origin of periclinal chimeras shows that cells at the apex of meristems do divide and form tissues.

3. Some plants have discrete and therefore stable germ layers as predicted by the tunica-corpus theory. Others have unstable germ layers.

4. Stable germ layers can exist even in meristems without a true tunica : the germ layers are then usually unstable, causing periclinal chimeras to become, first, mericlinal, and then non-chimerical.

5. The normal instability of mericlinal chimeras, predicted by the tunica-corpus theory, is absent in meristems whose cells divide only in one of the longitudinal planes.

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REVIEWS

METHODS OF PLANT BREEDING. 2nd Ed. H. K. Hayes, F. R. Immer and D. C. Smith. London : McGraw-Hill. 1955. Pp. 551+lx. 64s.

This is the second edition of Hayes and Immer's *Methods of Plant Breeding* first published in the United States some fourteen years ago. The revision, made necessary by advancing knowledge, has been undertaken by Dr Hayes in conjunction with Dr Smith whose name is therefore now added to the list of authors. Even though it is over ten years since he died, Dr Immer's name is retained, as a tribute to the value of the contribution he made when the book was first written.

The second edition shows a material expansion over the first, though the changes are by no means all simple additions. Rearrangements and even some deletions have also occurred. Among the additions are new chapters on heterosis, cotton and sorghum breeding, forage crops, and heritability. Sugar-cane breeding has been introduced and breeding for resistance to insect damage has been given fuller treatment. At the same time certain earlier chapters on the cereals have been combined into one. The extensive and useful account of statistical methods and field test design has been completely rearranged and to some extent amplified, though the description of lattice designs for testing large numbers of varieties has been perhaps a little surprisingly omitted.

These various alterations, however, have left unaffected the essential nature of the book as a compendium of information on plants and especially methods for the student of plant-breeding. As such it must be judged successful. No student who reads it at all conscientiously can fail to acquire a sound working knowledge of the problems facing the breeder and the methods for coping with them in our chief agricultural crop plants. He can turn back too and refresh his memory of the breeding techniques from the detailed schedules and diagrams in which the different operations are set out in various parts of the book. He can quickly look up the ways of carrying out at least most of the statistical operations which face him and he will find a most useful set of statistical tables collected at the end of the text. There is also a somewhat limited glossary of terms and an extensive collection of references, mainly American, for further and more specialised reading.

The early chapters set out to give an account of the role and of the genetical basis of plant-breeding, but they are disappointingly disjointed in both coverage and treatment. Nor are they always as up-to-date in the information they provide as one might reasonably expect. No doubt the student should already have a basic knowledge of genetics on which to build when he turns to plant-breeding, but a more systematic development of genetical principles as they apply to the work of the breeder would surely have been worth while. It is also disappointing to find no account in the chapter on seed production of the information which has come available, particularly during the past decade, about the factors determining pollen contamination and hence isolation requirements during the multiplication of seed. Neither the plant-breeder's task not the application of genetical principles stops short when the first seed of a new variety is produced.

These considerations should not, however, blind us to the book's virtues. Within its own limits it meets a real need and it will continue to be used both widely and profitably by students, and, we may venture to think, sometimes by more mature plant breeders also. The new edition is well produced and indeed comparison in this respect with its predecessor shows how far we have come from wartime and post-war shortages. We can also see how far we have moved in another respect, for the price in Great Britain has more than doubled. KENNETH MATHER.

FACTS OF LIFE. By C. D. Darlington. Allen and Unwin. Pp. 467. 40s.

Excuse for the appearance of a further review of Professor Darlington's book so long after publication may be found in the magnitude of the task, and in the importance of assessing its message.

The first two sections of the book are directed to setting out modern knowledge of reproduction and heredity by the historical method. The development of accurate notions of heredity has been a difficult matter as compared with the ease with which the physical sciences have progressed. Both the means of investigation and the logic of experimentation and inference had to reach a somewhat sophisticated level before decisive results could be got. But there is another reason, in some ways more serious (especially in that it still has implications for the present day). Darlington finds this in what may broadly be called " superstition " or a confusion between biology and morals. The facts of conception, birth and inheritance touch us all both more deeply and also more familiarly than, say, do the Gas Laws. One result is that vulgar ideas are hard to dislodge " Everybody already knows too much about it ". Another result is that all these topics are emotionally charged and a Freudian " resistance " makes us hold to preconceptions, which are often misconceptions. This happens in part by taboo ; in part because we are reluctant to follow out ideas to the point where we might need to question the relevant laws of Church or State : our own personal interpretation (sustaining our morale in daily life) of our relationship as individuals to the rest of Society is often based on suppositions which concern genetics.

Darlington's history of genetics thus has several themes. It disentangles the interactions between prejudice and discovery. It is an essay in scientific method and also in the sociology of science. And it analyses the philosophic bases of the notions of the great theorists.

We now reach modern genetics, with material genes and chromosomes, and a materialist theory of life and inheritance. The author now considers the general consequences of this theory.

In the first place, it should be noticed that Darlington renders a service to epistemology by his analysis of the notions involved in " indeterminacy ". Instead of this hackneyed and multivalent word, he uses " uncertainty " as descriptive of the random element in biological processes. This random element no more derives from a lack of causality than do the roulette wheels at Monte Carlo. It is a determinate consequence of understood mechanisms.

On this basis the mechanism of heredity though material is not simply determinate. Assortment and recombination imply a large scale uncertainty in inheritance. This uncertainty provides variation. Selection operates on variation. " Evolution does not arise from a property of progress or

improvement inherent in life ". It arises from the selection of uncertainty. Thus the relation between determinism and uncertainty on the species level is a dialectical one, and the continued maintenance of genetic uncertainty in the scheme of things must be ascribed to this relationship. However, in the case of the individual life-history once the zygote is formed, uncertainty is almost ruled out. Development of embryos is strictly regulated and with equal environments uncertainty enters only in the form of rare errors, or minor variation which is unimportant to the functioning of the organism.

The rest of the book, except for an Interlude on the Lysenko scandal, is concerned with applying genetical findings to the serious problems of human life. If we have subscribed thus far to Darlington's statement of biological realities (and if pressed most professional geneticists would, like myself, feel bound to do so), we must acknowledge that the inheritance of acquired characters is exceedingly rare. The germ plasm of a stock is not changeable by nutrition, education or social conditioning *per se* but only in so far as these environmental factors operate selectively. Thus environmental influences, physical or intellectual or emotional, directly affect at most the personal development of the individual. Their indirect effect is confined to selection modifying the germ plasm of a stock. Thus two lines of enquiry are marked out in the human field. To what extent has selection operating in prehistoric and historic times produced important genetic differences between human groups? In what proportions do genotype and environment respectively contribute to an individual's personality?

Taking the second question first, the study of twins shows us the cardinal importance of genotype. Our potentialities are given at conception. Darlington, however, does not minify the role of environment. He points out that in the human field it is not obvious (even if at first sight it may seem so) " which is the necessary or favourable environment for a particular genotype. . . . Environments no more than genotypes are to be arrayed in an objective scale of values. Our judgment is as much warped by our own prejudices, our own snobbery, in assessing the one as the other ". There is a warning here that the evolution of the Welfare State may have unexpected effects on the character of its social classes.

Is there anything also in the personality besides the genotype and its reaction to environment? Is there Free Will? Darlington comes down against it, and I agree with him. This view of the matter does not in any way diminish the imperative to maintain our responsibility for our acts. Rather it should increase it. The genetic findings, on the other hand, make nonsense of the punitive approach to errors, crimes and deviant morals. The notion of punishment as distinct from disciplinary control and training is one which will have to go from any society that aims at regulating its affairs with scientific understanding.

Many will agree similarly with the view that " It is now time that the State based its intervention in marriage not on confused myths and traditions but on authentic knowledge and explicit reasons. A society which is bound to maintain itself by sexual reproduction is more likely to survive if it seeks to understand the facts of life and apply that understanding to its customs and its laws ".

But now Darlington poses us other riddles. What he has to say is a

corrective to the repudiation of heredity in the social sciences. This repudiation may be dangerous, not only in day-to-day problems such as delinquency, but in the long-term problems of population change.

One of the end products of the book is a " biological interpretation of history " written with a proper (and I think entirely justified) respect for Marx. Darlington is careful to stress that an interpretation is not a " formula, or the demonstration of a simple pattern which will describe the course of history and predict the future ". The biological interpretation reinstates the submerged part of the genetic iceberg as a leading agent in social change. The top of the iceberg is the complex of economic, political and social conditions. This superstructure has of course its own dynamic and is to a certain extent autonomous. The genetic substructure, however, conditions historical events in various ways. One mode of action is to introduce uncertainty into history. Chance throws up from time to time the rare genotype. If also the historical situation is particularly unstable we get the conjunction of the man and the hour : Lenin comes to the Finland Station.

There is a discussion of the origin of social class. Classes both for Marx and Darlington have evolved for positive social reasons. Each class owes its position to the fact that at one time at least it performed some function in society. Marx would say that as the relations of production change, the role of the ruling classes may become obsolete or even negative. This contradiction between the class structure and the economic realities results in decline or crisis. Darlington would add " The fitness of a race or class for its allotted work must always be changing, for neither genotype nor environment can be kept stable. Genetic recombination changes all groups, especially small and isolated groups such as those empirically described as imperial races and governing classes. They derive their dominant position from the fitness of their genetic character to the conditions they find or make for themselves. They also owe their later downfall, . . . to changes in their genetic character, or in their external situation, or more usually in both ". And later he says, " It is the purpose of all governing classes to protect themselves against natural selection. The intelligence they may use in doing so, and the lengths they will go in sacrificing their society as a whole to their own interests to avoid their own destruction are both variable. The examples of Spain, France and England show a gradient, . . . between situations in which Marx is largely right and largely wrong, a gradient which is itself genetically determined ".

I have tried to sketch Darlington's principal themes according to my own understanding of what he is saying. I have had to summarise his conclusions and I have not done justice to the complexity of the argument. This complexity is inherent in the facts. It is an achievement to have carried it through, as he has done (in a not especially lengthy book) without clarity or interest failing at any point. An heroic book of this sort is bound to generate the most diverse reactions in its readers, or even in the same reader. It will *épater le bourgeois*, and the *anti-bourgeois* also. No doubt a minority of readers will feel it is all true enough, but what then, is it not all a truism ? This will be because they have missed one of the points. It is one thing to receive a theory, it is another to take it into one's self so that each problem is examined in its light. A. R. G. Owen.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTY-SECOND MEETING of the Society, held on 10th NOVEMBER 1956, at the GALTON LABORATORY, UNIVERSITY COLLEGE, LONDON

TIME OF ACTION OF DOMINANT LETHALS IN MAMMALS

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Dominant lethal mutations induced in the sperm of a mouse manifest themselves in two ways : as fertilised eggs which fail to implant, and as implants which fail to develop. The relative frequencies of these two classes, usually described as pre-implantation and post-implantation deaths, may vary within wide limits.

A preliminary study has been made of the factors responsible for this variation, such as maternal effects, timing of insemination, amount and vitality of sperm, and nature of the dominant lethal mutation itself. Though only the last of these is a dominant lethal phenomenon, the others are all obstacles to the proper interpretation of dominant lethal studies.

EFFECT OF PARENTAL ENVIRONMENT ON OFFSPRING CHARACTER IN FLAX

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All eight combinations of N, P and K (parental) fertiliser treatments were applied to 40 flax plants of the variety Stormont Cirrus. The seed collected from them varied in weight but germinated evenly the following year when the progeny were grown in all 16 combinations of N, P, K and Ca (offspring) fertiliser treatments. The 640 plants grown were cut at ground level at maturity and weighed. The parental treatments (as well as the offspring treatments) produced large and significant differences in weight, some treatments producing two or three times the weight produced by others. These differences appear to be in no way diminished in the second generation growing this year. Small, but significant, interactions occurred between parental and offspring treatments, plants responding more to phosphate, for example, if their parents received phosphate. Responses of this magnitude, due to changes in the cytoplasm, may be obtained only when the parental treatments are applied in poor growing seasons of damp, cool conditions with little sun, but once produced they can persist through a good growing season to appear again the following year even though the seed sown then is of high and uniform weight.

POLYGENIC INHERITANCE OF ASCOSPORE SIZE IN *NEUROSPORA CRASSA*

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Selection for large ascospores in *Neurospora crassa* has increased the mean size from 14·1 microns × 26·9 microns to 16·8 microns × 35·8 microns in the course of 16 generations. The greater part of this increase in size is due to a steadily

increasing frequency of asci containing 4 ascospores of about twice normal size instead of 8 normal ascospores. The number of asci containing 8 mature ascospores has steadily decreased but selection has also increased the size of the more or less normal ascospores.

All the asci produced by reciprocal crosses of strains from the sixteenth generation of selection with stock wild-types, have been of the normal 8-spored type. Thus the eight-spored character is " dominant " and the size of ascospore and type of ascus apparently depend chiefly on nuclear and not cytoplasmic characters. There is some suggestion of a cytoplasmic effect however since crosses with the stock wild-type as the maternal parent show a slightly, but probably significantly, larger mean ascospore size than the reciprocals.

A range of mean ascospore size found in both F1 crosses and in backcrosses indicates that a considerable number of genes affecting ascospore size differentiate the selected and wild-type strains. The steady response to selection also suggests a polygenic character.

NEW MATING TYPES IN *ESCHERICHIA COLI*

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Among many micro-organisms there exists a wide diversity of mating types, even within a single species. In *Escherichia coli* only three types have hitherto been described (F−, F+, Hfr), all within strain K12. These differ in their mating behaviour ; in their ability to infect other bacterial strains with a transmissible mating-type agent ; and in their susceptibility to infection by such an agent. Other strains of *E. coli* were studied with respect to these criteria, and several new mating types have been discovered.

One of these strains, WG3, possesses an agent " F3 " which was compared with the K12 agent " F12." Suitably marked F− strains, derived from K12, were infected with the F3 and F12 agents, and the F3+, F12+, and F− were crossed in all possible combinations. The agents differ in the degree of fertility they confer, and in their inheritance among progeny (F3 is not invariably inherited). Most notably, the frequencies of genetic recombination in each region of the genome were greatly dependent on the agent used. The " F polarity " characteristic of F12 is not found in the case of F3, and the " donor-receptor " concept of mating behaviour applied only to F12+ × F− crosses.

BACTERIAL MOTILITY : AN ANALYSIS OF VARIATION AMONGST CELLS OF IDENTICAL GENOTYPE SHARING A COMMON " UNIFORM " ENVIRONMENT

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A clone of a non-motile *Salmonella* strain grown in a uniform environment (*e.g.* a shaken broth culture) may contain some motile individuals, not mutants, since their progeny are non-motile.

These motile cells may result from the transmission, through many generations, of non-replicated ancestral particles : either flagella, possession of one of which confers motility ; or, in abortive transduction, of a supernumerary gene for synthesis of flagella, introduced into the ancestor.

In some strains, however, a minority of cells acquire, apparently at random, a transient ability to synthesise flagella. The resulting differences between cells in

ability to synthesise flagella are not due to differences in their genotypes, nor to detectable differences in their environments. Such "residual" variation may, speculatively, be attributed to such minor environmental differences as may occur within a uniform liquid. This deterministic hypothesis should be accompanied by a probabilistic description, *viz.* that knowledge of genotype, parental phenotype, and environment, with the greatest accuracy now feasible, permits prediction, for an individual, only of the *probability* of a particular phenotype.

Spontaneous mutation occurring "at random" is a special case in which residual variation affects a genetic, instead of a somatic, cell component.

THE CONTROL OF MAMMALIAN SEX RATIOS

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Weir has reported that the sex ratio of a strain of mice depends on the blood *p*H of the sires. There is evidence from several sources that this principle extends to other mammals, including Man, cattle, sheep and rats. It also appears that induced changes of *p*H may be as effective as the genetically determined deviations in Weir's selected lines of mice. The equilibration of a gene which affects the sex ratio (but only when present in one of the sexes) presents a new problem in population genetics.

THE GENETICS OF ESSENTIAL HYPERTENSION

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Evidence has recently been accumulating which points to two conclusions (1) that there is no qualitative difference between hypertension and normality, hypertensive subjects representing the positive tail of the continuous frequency distribution of arterial pressures (2) that over the whole range of pressures, including hypertension, the genetic component is represented by a correlation of a little more than 0·2 between first degree relatives. The most recent work, including parallel studies by Miall and Oldham, shows that the same regression applies to several different samples of the general population and to relatives of hypertensives. When a possible confusing factor, arm-circumference (or obesity) is held constant, the genetic resemblances are increased. The frequency distribution of arterial pressures turns out to be closely lognormal, both in the general population and in relatives of hypertensives, so providing evidence against bimodality.

AN IMPROVEMENT IN THE TASTER-NONTASTER CLASSIFICATION

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1. The sensitivity of a person to PTC-solutions depends on the genetical constitution at the T locus and on other general factors for which the sensitivity to solutions of quinine or brucine can provide some measurement.

2. Brucine does not show any bimodality of thresholds.

3. By considering the quinine threshold as well as age and sex of a person the PTC threshold can be so corrected as to arrive at a better genotypical classification. Corrections appropriate for samples from European populations are suggested in the form of a diagram.

COLOUR POLYMORPHISM IN THE EARTHWORM *ALLOLOBOPHORA CHLOROTICA*

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and

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Two types of *Allolobophora chloratica* exist in one of which porphyrins and a biliverdin-like substance accumulate during the individual's lifetime, rendering it green. Populations of the green form occur in grassland and pure pink populations in woodland gardens and certain types of grassland. Mixed populations were found in cultivated fields and in grassland which had been previously cultivated. Evidence is presented which seems to indicate that the possession of the green pigmentation is controlled by a single recessive Mendelian factor. Various possibilities, among them predation, are discussed which could account for the ecological distribution of the green and pink populations.